

# From Basic to Clinical Immunology

Vladimir V. Klimov

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**Vladimir V. Klimov**  
Clinical Immunology and Allergy Department  
Siberian State Medical University  
Tomsk, Russia

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Tu se' omai al purgatorio giunto:  
vedi là il balzo che 'l chiude dintorno:  
vedi l'entrata là 've par disgiunto.

You have finally arrived in Purgatory:  
There you can see the cliffs which wraps around it;  
There is the entrance, where there is a split.

Dante Alighieri. La Divina Commedia.  
Purgatorio. Canto IX, 49-51

# Preface

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Why is immunology so important? The immune system has involvement in almost all fields related to health and disease. Infections continue to confront human health and well-being on a global scale. Inflammation contributes to the lung, heart and joint diseases, and diabetes mellitus; cancers have to evade immune surveillance, and immune dysregulation leads to allergies that are increasingly prevalent across the world. Only improved understanding of the mechanisms by which microbes, allergens, and tumor cells cause disease will result in the development of diagnostic, therapeutic, and preventative strategies to combat this threat.

However, we are only beginning the voyage of immunology, and there is much we still need to research and understand. The study of basic immunology may provide students with an opportunity to relate the findings of fundamental scientific investigations to clinical problems. Since immunology is a very complex science, this manual has been arranged in a simplistic yet logical manner so that students could perceive basic principles of the subject and, at the same time, understand important particularities.

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**Vladimir V. Klimov**  
Tomsk, Russia

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## About the Author

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**Vladimir Klimov, MD, PhD, DSc**

is the head of Siberian State Medical University's Immunology and Allergy Department. He is the author of the multimedia course "Basic Immunology Overview," which was published online in the late 1990s and became popular among students and physicians throughout the world. For many years, Prof. Klimov contributed to immunology education internationally with great enthusiasm. This manual was written at the interface of fundamental and clinical immunology. "What is clinical immunology? It is a medical science about the commensal germs reactivation, breakdown of natural tolerance, and disorders in cancer containment," says Prof. Klimov.

[klimov@mail.tomsynet.ru](mailto:klimov@mail.tomsynet.ru)

# List of Abbreviations

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<b>AAMPs</b>	Allergen-associated molecular patterns	<b>BLC</b>	B-lymphocyte chemoattractant, CXCL13
<b>AChR</b>	Acetylcholine receptor	<b>BRAK</b>	Breast and kidney-expressed chemokine, CXCL14
<b>ACTH</b>	Adrenocorticotrophic hormone	<b>BTK</b>	Tyrosine kinase (Bruton's) gene
<b>ADA</b>	Adenosine deaminase	<b>C1NH</b>	C1 inhibitor gene
<b>ADDC</b>	Antibody-dependent cellular cytotoxicity	<b>C2a, etc.</b>	Complement fragments
<b>ADH</b>	Antidiuretic hormone, vasopressin	<b>C3bBb</b>	Alternative pathway C3 convertase
<b>AFP</b>	$\alpha$ fetoprotein	<b>C3bBb3b</b>	Alternative pathway C5 convertase
<b>AHR</b>	A signaling molecule	<b>C4b2b</b>	Classical pathway C3 convertase
<b>AIDS</b>	Acquired immunodeficiency syndrome	<b>C4b2b3b</b>	Classical pathway C5 convertase
<b>AIM-2</b>	"Absent in melanoma 2," a part of ALR	<b>CAMs</b>	Cell adhesion molecules
<b>AIRE</b>	Autoimmune regulator gene	<b>cAMP</b>	Cyclic adenosine monophosphate
<b>AK2</b>	Mitochondrial adenylate kinase 2	<b>CARD</b>	Caspase activation and recruitment domain
<b>ALPS</b>	Autoimmune lymphoproliferative syndrome	<b>CCL</b>	A chemokine subfamily
<b>ALR</b>	AIM-2-like receptor	<b>CCP</b>	Cyclic citrullinated peptide
<b>APC</b>	Antigen-presenting cell	<b>CCR</b>	A receptor to CCL and other chemokines
<b>APECED</b>	Autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy	<b>CD</b>	Cluster of differentiation, a differentiation marker
<b>AR</b>	Activating receptor	<b>cDC</b>	Classical (conventional) dendritic cell, mDC
<b>ASC</b>	An adapter protein	<b>CEA</b>	Carcinoembryonic antigen
<b>ASIT</b>	Allergen-specific immunotherapy	<b>CFS</b>	Chronic fatigue syndrome
<b>ATM</b>	Ataxia-telangiectasia mutated gene	<b>CFSE</b>	Carboxyfluorescein succinimidyl ester, a fluorochrome
<b>ATP</b>	Adenosine triphosphate	<b>CgA</b>	Chromogranin A
<b>B2M</b>	$\beta_2$ microglobulin	<b>CGD</b>	Chronic granulomatous disease
<b>B7-1, B7-2</b>	Costimulatory molecules, counterreceptors for CD28 and CTLA-4	<b>cGMP</b>	Cyclic guanosine monophosphate
<b>BAFF</b>	B-cell activation factor	<b>CH</b>	Constant heavy domain
<b>BALT</b>	Bronchus-associated lymphoid tissue	<b>CL</b>	Constant light domain
<b>BAU</b>	Bioequivalent allergen unit	<b>CLA</b>	Cutaneous lymphocyte-associated antigen
<b>BCR</b>	B-cell receptor		

## List of Abbreviations

<b>CLIP</b>	A component of li chain	<b>DC</b>	Dendritic cell
<b>CLR</b>	C-type lectin receptor	<b>DGP</b>	Deamidated gliadin peptide
<b>CLS</b>	Capillary leak syndrome	<b>DHEA</b>	Dehydroepiandrosterone
<b>CMV</b>	<i>Cytomegalovirus</i>	<b>DN</b>	Double-negative (thymocytes)
<b>CNS</b>	Central nervous system	<b>DOCK8</b>	A signaling molecule
<b>ConA</b>	Concanavalin A (mitogen)	<b>DP</b>	Double-positive (thymocytes)
<b>COP</b>	CARD-only protein	<b>DPI</b>	Dry powder inhaler
<b>COPD</b>	Chronic obstructive pulmonary disease	<b>dsDNA</b>	Double-stranded DNA
<b>CpG</b>	Motif in a PAMP molecule	<b>dsRNA</b>	Double-stranded RNA
<b>CR</b>	Complement receptor	<b>DTaP-HepB-IPV</b>	Vaccine against diphtheria, tetanus, pertussis, hepatitis B, polio
<b>CRD</b>	Carbohydrate recognition domain	<b>DTaP-IPV/Hib</b>	Vaccine against diphtheria, tetanus, pertussis, polio, <i>H. influenzae</i> type b infection
<b>CREST</b>	Syndrome composed of calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia		
<b>CRISPR/Cas9 technique</b>	A novel gene editing technology (e.g., for vaccine development)	<b>EBV</b>	<i>Epstein-Barr Virus</i>
<b>CSF</b>	Colony-stimulating factor	<b>ECA</b>	Endothelial cell antigen
<b>CTACK</b>	Cutaneous T-cell-attracting chemokine, CCL27	<b>ECM</b>	Extracellular matrix
<b>CTLA-4</b>	Cytotoxic T lymphocyte-associated protein-4, CD152	<b>ELC</b>	EB1 ligand chemokine, exodus-3, CCL19
<b>CVID</b>	Common variable immunodeficiency	<b>ELISA</b>	Enzyme-linked immunosorbent assay
<b>CX3CL</b>	A chemokine subfamily	<b>ELISPOT</b>	A modification of ELISA
<b>CX3CR</b>	A receptor to CX3CL and other chemokines	<b>ENA-78</b>	Epithelial-derived neutrophil-activating peptide 78, CXCL5
<b>CXCL</b>	A chemokine subfamily	<b>Fab</b>	Fragment antigen binding
<b>CXCR</b>	A receptor to CXCL and other chemokines	<b>Fas</b>	An apoptosis receptor, CD95
<b>CYBB</b>	Gene encoding phagocyte NADPH oxidase (phox) complex	<b>FasL</b>	Ligand for Fas, CD178
<b>DAF</b>	Decay-accelerating factor, CD55	<b>Fc</b>	Fragment crystallizable
<b>DAMPs</b>	Damage-associated molecular patterns	<b>FCFM</b>	Flow cytofluorometry
		<b>FCGR3A</b>	CD16 gene
		<b>FcR</b>	Fc receptor
		<b>fDC</b>	Follicular dendritic cell

<b>FERMT3</b>	Gene encoding an intracellular protein, which interacts with $\beta$ integrins	<b>HepA</b>	Vaccine against hepatitis A
<b>FEV1</b>	Forced expiratory volume in 1 second	<b>HepB</b>	Vaccine against hepatitis B
<b>FGF</b>	Fibroblast growth factor	<b>HEV</b>	High endothelial venules
<b>FITC</b>	Fluorescein isothiocyanate, a fluorochrome	<b>HHV-8</b>	<i>Human Herpes Virus 8</i>
<b>FoxP3</b>	A transcription factor	<b>Hib</b>	Vaccine against <i>H. influenzae type b</i> infection
<b>FVC</b>	Full (forced) vital capacity of lungs	<b>HIN200</b>	Domain, which consists of hematopoietic expression, IFN-inducible and nuclear localization of 200 amino acids
<b>G6PD</b>	Glucose-6-phosphate dehydrogenase	<b>HIV</b>	<i>Human Immunodeficiency Virus</i>
<b>GABA</b>	$\gamma$ aminobutyric acid	<b>HLA</b>	Human leukocyte antigens, human histocompatibility complex
<b>GAD</b>	Glutamate decarboxylase	<b>HPA</b>	Hypothalamus–pituitary–adrenal (axis)
<b>GALT</b>	Gut-associated lymphoid tissue	<b>HPV</b>	<i>Human Papilloma Virus</i>
<b>GATA-3</b>	A signaling molecule	<b>HPV2, HPV4, HPV9</b>	Vaccines against <i>Human Papilloma Virus</i> infection
<b>GBM</b>	Glomerular basement membrane	<b>HSV</b>	<i>Herpes Simplex Virus</i>
<b>GCP-2</b>	Granulocyte chemotactic protein-2, CXCL6	<b>5-HT</b>	5-hydroxytryptamine, serotonin
<b>G-CSF</b>	Granulocyte colony-stimulating factor	<b>hTM5</b>	Human tropomyosin isoform 5
<b>GI</b>	Gastrointestinal tract	<b>HZV</b>	<i>Herpes Zoster Virus</i>
<b>GLYCAM-1</b>	Glycosylation-dependent cell adhesion molecule-1, a mucin-type CAM	<b>ICAM-1,-2,-3</b>	Intercellular adhesion molecules
<b>GM-CSF</b>	Granulocyte-macrophage colony-stimulating factor	<b>ICOS</b>	A costimulatory molecule
<b>GRB2</b>	An adaptor protein	<b>IEL</b>	Intraepithelial lymphocytes, $\gamma\delta$ T cells
<b>GRO<math>\alpha</math></b>	Growth-regulated protein- $\alpha$ , CXCL1	<b>IFN<math>\alpha</math>,-<math>\beta</math>,-<math>\gamma</math></b>	Interferons
<b>GRO<math>\beta</math></b>	Growth-regulated protein- $\beta$ , MIP-2 $\alpha$ , CXCL2	<b>IFNGR1</b>	IFN $\gamma$ RI gene
<b>GRO<math>\gamma</math></b>	Growth-regulated protein- $\gamma$ , MIP-2 $\beta$ , CXCL3	<b>IGAD-1</b>	IgA deficiency locus-1
<b>GVHD</b>	Graft-versus-host disease	<b>IGF-1</b>	Insulin-like growth factor 1
<b>H</b>	Heavy (chain)	<b>IGH</b>	Locus of immunoglobulin H chain genes
<b>H1-H4</b>	Histamine receptors	<b>IGK</b>	Locus of immunoglobulin $\kappa$ chain genes
<b>HCC-1</b>	Hemofiltrate CC chemokine-1, CCL14	<b>IGL</b>	Locus of immunoglobulin $\lambda$ chain genes
<b>HE4</b>	Human epididymis protein 4		
<b>HEP</b>	Histamine equivalent prick test (unit of allergen activity)		

## List of Abbreviations

<b>IIV</b>	Vaccine against seasonal influenza (flu)	<b>LILR</b>	Leukocyte immunoglobulin-like receptor
<b>IL</b>	Interleukin	<b>LKM</b>	Liver/kidney microsomes
<b>IL1ra</b>	IL1 receptor antagonist	<b>LMP-2, LMP-7</b>	Components of the proteasome
<b>ILC</b>	Innate lymphoid cell	<b>LPS</b>	Lipopolysaccharide
<b>IgM, IgG, IgA, IgE, IgD</b>	Immunoglobulins or antibodies	<b>LRR</b>	Leucine-rich domain
<b>IP-10</b>	IFN $\gamma$ -induced protein-10, CXCL10	<b>LTB4</b>	Leukotriene B4
<b>IPEX</b>	X-linked immunodysregulation, polyendocrinopathy, enteropathy syndrome	<b>LTC4</b>	Leukotriene C4
<b>IR</b>	Inhibitory receptor	<b>LTH</b>	Lactotropic hormone, prolactin
<b>I-TAC</b>	IFN-inducible T-cell $\alpha$ chemoattractant, CXCL11	<b>LTi</b>	Lymphoid tissue inducer cell
<b>ITAM</b>	Immunoreceptor tyrosine-based activation motif	<b>LTT</b>	Lymphoblast transformation test
<b>ITGB2</b>	CD18 gene	<b>Lyn</b>	A tyrosine kinase
<b>ITIM</b>	Immunoreceptor tyrosine-based inhibitory motif	<b>M1</b>	Type 1 macrophage
<b>iTreg</b>	Induced T-regulatory cell	<b>M2</b>	Type 2 macrophage
<b>IVIG</b>	Intravenous immunoglobulin (administration)	<b>MAC</b>	Membrane attack complex, C5b6789...9
<b>JAK</b>	Janus (tyrosine) kinase	<b>MadCAM-1</b>	Mucosal vascular addressin cell adhesion molecule-1
<b>Ki-67</b>	A nuclear protein, a marker of the cell proliferation assay	<b>MALT</b>	Mucosa-associated lymphoid tissue
<b>KIR</b>	Killer immunoglobulin-like receptor	<b>MBL</b>	Mannose-binding lectin
<b>KLRG1</b>	Killer lectin-like receptor G1	<b>MBP</b>	Myelin basic protein
<b>L</b>	Light (chain)	<b>MCP-1</b>	Macrophage chemoattractant protein-1, CCL2
<b>LAD</b>	Leukocyte adhesion deficiency	<b>MCP-2</b>	Macrophage chemoattractant protein-2, CCL8
<b>LAT</b>	An adaptor protein	<b>MCP-3</b>	Macrophage chemoattractant protein-3, CCL7
<b>LC-1</b>	Liver cytosol antigen 1	<b>M-CSF</b>	Macrophage colony-stimulating factor
<b>Lck</b>	A tyrosine kinase	<b>mDC</b>	Myeloid (classical, conventional) dendritic cell, cDC
<b>LFA-1</b>	Lymphocyte function-associated antigen-1, an integrin	<b>MDI</b>	Meter-dose inhaler
		<b>MDSC</b>	Myeloid-derived suppressor cell
		<b>MECL</b>	A component of the proteasome
		<b>MEC</b>	Mucosa-associated Epithelial Chemokine, CCL28

<b>MEFV gene</b>	Gene for pyrin	<b>NET</b>	Neutrophil extracellular trap during NETosis
<b>MenACWY, MenB, MPSV4</b>	Meningococcal vaccines	<b>NFAT</b>	A transcription factor
<b>MenCY-Hib</b>	Vaccine against meningococcal and <i>H. influenzae</i> type b infections	<b>NF-<math>\kappa</math>B</b>	A transcription factor
<b>MIG</b>	Monokine induced by IFN $\gamma$ , CXCL9	<b>NK</b>	Nature killer cell
<b>MIP-1<math>\alpha</math></b>	Macrophage inflammatory protein-1 $\alpha$ , CCL3	<b>NKG2/CD94</b>	Natural killer (lectin-like) receptor G2/CD94
<b>MIP-1<math>\beta</math></b>	Macrophage inflammatory protein-1 $\beta$ , CCL4	<b>NKT</b>	Nature killer T cell
<b>MIP-2<math>\alpha</math></b>	Macrophage inflammatory protein-2 $\alpha$ , GRO $\beta$ , CXCL2	<b>NLR</b>	NOD-like receptor
<b>MIP-2<math>\beta</math></b>	Macrophage inflammatory protein-2 $\beta$ , GRO $\gamma$ , CXCL3	<b>NLRP3</b>	An inflammasome
<b>MMR</b>	Vaccine against measles, mumps, rubella	<b>NLRP3 gene</b>	Gene for cryopyrin
<b>MMRV</b>	Vaccine against measles, mumps, rubella, varicella	<b>NOD</b>	Nucleotide-binding oligomerization domain
<b>mRNA</b>	Messenger RNA	<b>NSAIDs</b>	Nonsteroid anti-inflammatory drugs
<b>MSH</b>	Melanocyte-stimulatory hormone	<b>NSE</b>	Neuron-specific enolase
<b>MTS, MTT</b>	Dyes for the colorimetric proliferation assays	<b>nTreg</b>	Natural T-regulatory cell
<b>MuSK</b>	Muscle-specific receptor tyrosine kinase	<b>NU-ELISA</b>	A modification of ELISA
<b>MyD88</b>	An adapter protein for TLR signaling	<b>OAS</b>	2',5'-oligoadenylate-synthetase
<b>MZ</b>	Marginal zone (in the spleen)	<b>p56<sup>lck</sup></b>	A tyrosine kinase
<b>NACHT</b>	A central domain in NLRs	<b>PAF</b>	Platelet-activating factor
<b>NALT</b>	Nasal-associated lymphoid tissue	<b>PALS</b>	Periarteriolar lymphoid sheaths (in the spleen)
<b>NAP-2</b>	Neutrophil-activating peptide-2, CXCL7	<b>PAMPs</b>	Pathogen-associated molecular patterns
<b>NBN</b>	Nibrin gene important for cell cycle	<b>PCV13, PPSV23</b>	Pneumococcal vaccines
<b>nBreg</b>	Natural B-regulatory cell	<b>pDC</b>	Plasmacytoid dendritic cell
<b>NBT</b>	Nitroblue tetrazolium	<b>PDGF</b>	Platelet-derived growth factor
<b>NCA</b>	Neutrophilic cytoplasmic antigens	<b>PE</b>	Phycoerythrin, a fluorochrome
<b>NCK</b>	An adaptor protein	<b>PECAM-1</b>	Platelet-endothelial cell adhesion molecule-1, CD31
<b>NCR</b>	Natural cytotoxicity receptor	<b>PGD2</b>	Prostaglandin D2
		<b>PHA</b>	Phytohaemagglutinin (mitogen)
		<b>pIgR</b>	Polymeric Ig receptor
		<b>PKR</b>	Protein kinase R
		<b>PLC<math>\gamma</math>1, -2</b>	Phospholipase C $\gamma$
		<b>PMN</b>	Polymorphonuclear leukocytes
		<b>PNU</b>	Protein nitrogen unit (of allergen)

## List of Abbreviations

<b>POP</b>	PYD-only protein	<b>SLC</b>	Secondary lymphoid tissue chemokine, Exodus-2, CCL21
<b>PRRs</b>	Pattern recognition receptors	<b>SLC35C1</b>	Gene encoding a GDP-fucose transmembrane transporter
<b>PSA</b>	Prostate-specific antigen	<b>SLE</b>	Systemic lupus erythematosus
<b>PSGL-1</b>	P-selectin glycoprotein ligand-1, a mucin-type CAM	<b>SLP76</b>	An adaptor protein
<b>PWM</b>	Pokeweed mitogen	<b>SLP/BLNK</b>	An adaptor protein
<b>PYD</b>	Pyrin domain	<b>SP</b>	Single-positive (thymocytes)
<b>qPCR</b>	Quantitative polymerase chain reaction	<b>SP-A, SP-D</b>	Surfactant proteins
<b>RAAS</b>	Renin-angiotensin-aldosterone system	<b>ssRNA</b>	Single-stranded RNA
<b>RAG-1, RAG-2</b>	Recombination-activating genes	<b>STAT3</b>	A transcription factor
<b>RANTES</b>	Regulation on activation, normal T-cell expressed and secreted, CCL5	<b>Syk</b>	A tyrosine kinase
<b>RIA</b>	Radioimmunoassay	<b>T3</b>	Triiodothyronine
<b>RIG-1</b>	Retinoid acid-inducible gene-1 for a part of RLP	<b>T4</b>	Thyroxine
<b>RLP</b>	RIG-1-like receptor	<b>TALT</b>	Tube-associated lymphoid tissue
<b>ROR-<math>\alpha</math>, ROR-<math>\gamma</math>t</b>	Signaling molecules	<b>TAM</b>	Tumor-associated macrophage
<b>ROS</b>	Reactive oxygen species, oxygen radicals	<b>TAN</b>	Tumor-associated neutrophil
<b>RT-PCR</b>	Reverse transcription polymerase chain reaction	<b>TAMPs</b>	Tumor-associated molecular patterns
<b>RV1, RV5</b>	Vaccine against rotavirus infection	<b>TAP-1, TAP-2</b>	Transporters associated with antigen processing
<b>SAA</b>	Serum amyloid A	<b>T-bet</b>	A signaling molecule
<b>SALT</b>	Skin-associated lymphoid tissue	<b>T<sub>CM</sub></b>	Central memory T cell
<b>SC</b>	Secretory component of secretory IgA	<b>TCR</b>	T-cell receptor
<b>SCDF-1</b>	Stromal cell-derived factor-1, CXCL12	<b>TECK</b>	Thymus-expressed chemokine, CCL25
<b>SCID</b>	Severe combined immunodeficiency	<b>T<sub>EM</sub></b>	Effector memory T cell
<b>SDS</b>	Sodium dodecyl sulfate	<b>Tfh</b>	Follicular helper T cell
<b>SIRS</b>	Systemic inflammatory response syndrome	<b>Tfr</b>	Follicular regulatory T cell
<b>SLA</b>	Soluble liver antigen	<b>TGF<math>\beta</math></b>	Transforming growth factor- $\beta$
		<b>Th</b>	Helper T cell
		<b>TIR</b>	Toll/IL1 receptor, a part of TLR
		<b>TLR</b>	Toll-like receptor
		<b>TNF<math>\alpha</math>, TNF<math>\beta</math></b>	Tumor necrosis factors
		<b>TRAD</b>	Locus of TCR $\alpha$ and $\delta$ chain genes

<b>TRB</b>	Locus of TCR $\beta$ chain genes	<b>VL</b>	Variable light domain
<b>TRG</b>	Locus of TCR $\gamma$ chain genes	<b>VLA-4</b>	Very late activation antigen-4, an integrin
<b>Tr1</b>	Type 1 regulatory T cell	<b>VLP</b>	Viruslike particle, a principle of the vaccine formation
<b>TRIF</b>	An adaptor protein for TLR signaling	<b>WASP</b>	Wiskott-Aldrich syndrome protein
<b>TSH</b>	Thyroid-stimulating hormone, thyrotropin	<b>WBC</b>	White blood cells
<b>tTG</b>	Tissue transglutaminase	<b>WHO</b>	World Health Organization
<b>uNK</b>	Uterine NK cell	<b>XCL</b>	A chemokine subfamily
<b>VAR</b>	Vaccine against varicella	<b>XCR</b>	A receptor to XCL and other chemokines
<b>Vav</b>	A signaling molecule	<b>Y</b>	A CLR's domain
<b>VCAM-1</b>	Vascular cell adhesion molecule-1, CD106	<b>Zap70</b>	A tyrosine kinase
<b>VDJC</b>	Immunoglobulin and TCR gene clusters		
<b>VH</b>	Variable heavy domain		

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- Video 1.2** Immunoglobulins
- Video 4.1** Simple B-cell-mediated response
- Video 4.2** Advanced B-cell-mediated response
- Video 4.3** CD4+ T-cell-mediated response
- Video 4.4** CD8+ T-cell-mediated response
- Video 4.5** Antigen processing



# Functional Organization of the Immune System

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**Electronic Supplementary Material** The online version of this chapter ([https://doi.org/10.1007/978-3-030-03323-1\\_1](https://doi.org/10.1007/978-3-030-03323-1_1)) contains supplementary material, which is available to authorized users.

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## Learning Objectives

*Knowledge.* Upon successful completion of the chapter, students should be able to:

1. Draw the functional organization of immune system.
2. Distinguish between antigens and “molecular patterns.”
3. Name and describe two main mechanisms of the immune system.
4. List the molecules of immune system and their functions.
5. Know the structure and functions of antibodies, B-cell receptors (BCRs), T-cell receptors (TCRs), and human leukocyte antigens (HLA).
6. Briefly summarize the basic facts about pattern recognition receptors (PRRs).
7. Be familiar with the cell adhesion molecules (CAMs).
8. Compare and contrast the functions of different cytokines and chemokines.
9. Outline the primary, secondary, and tertiary organs of the immune system.
10. Describe the cells of the immune system including T cells and B cells.
11. Explain the postulates of clonal selection theory.

*Acquired Skills.* Upon successful completion of the chapter, students should demonstrate following skills, including:

1. Interpret the knowledge related to the functional organization of the immune system.
2. Critically evaluate the scientific literature about structure and functions of the immune system.
3. Discuss the scientific articles from the current research literature to criticize experimental data and formulation of new hypotheses in basic immunology.
4. Attain a clear perception of the presented immunology definitions expressed orally and in written form.
5. Formulate the presented immunology terms.
6. Correctly answer quiz questions.

*Attitude and Professional Behaviors.* Students should be able to:

1. Have the readiness to be hardworking.
2. Behave professionally at all times.
3. Recognize the importance of studying and demonstrate a commitment.

## 1.1 Introduction

---

There is the explanation of such terms as “non-self,” “self,” and “former self” and what they matter in the immunology context. The reader can find a new idea related to the division of molecular patterns into PAMPs, AAMPs, DAMPs, and TAMPs. There is also the up-to-date description of structural features and functions of primary, secondary, and tertiary organs, cells, and molecules of the immune system. Clinical comments are accompanying almost every unit.

## 1.2 Antigens and “Patterns”

### Definitions

*Antigen* is a substance triggering the immune responses to constitute memory to this antigen. Antigens may be originated from “non-self,” “former self,” and even “self.” Antigens are categorized as *complete* and *incomplete (haptens)*, *T dependent* and *T independent*, and specified forms like *antigens of pathogens*, *allergens*, *tumor antigens*, *autoantigens*, etc.

*Molecular patterns* are low-molecular substances evoking the reactions of innate immunity with no memory. There are *pathogen-associated molecular patterns (PAMPs)*, *allergen-associated molecular patterns (AAMPs)*, *damage-associated molecular patterns (DAMPs)*, and *tumor-associated molecular patterns (TAMPs)*.

Any human body (“self”) exists within a hostile environment including microbes (“non-self”) and multicellular organisms (“non-self”). The external microbial environment and internal opportunistic germs, as well as even benign tumors, are not those places where any human body can know who to trust out here to survive. Fortunately, we have our immune system, which has evolutionarily known how to recognize “non-self,” “self,” and even “former self.” To understand, it is necessary to define the “non-self” and “self” at the molecular level in detail.

An antigen is a substance containing such information about “non-self,” “self,” and/or “former self,” which can trigger immune responses in the body to induce a very long and even lifelong memory to the event if it occurs. T-cell receptor (TCR) and B-cell receptor (BCR) can recognize antigens. Antigens of “self” are named autoantigens (or self-antigens), whereas tumor antigens present in fact “former self.” In the enlarged sense, it is currently estimated that the “universe of antigens” make up about  $10^{18}$  molecules in the environment. The antigens may be divided into complete and incomplete antigens (see ■ Table 1.1).

Any antigen as an *immunogen* may trigger an immune response, i.e., the interaction of many cell types of the immune system, which leads to the formation of new cell types destroying the antigen-containing pathogen and commonly keeping a memory about this event for a long time. Naturally, vaccines contain only immunogens. An antigen as a *tolerogen* triggers *immune tolerance*, another type of interaction of cells of the immune system. Alternatively, it results in the “specific immunological silence” when none is killed and no tissues are damaged.

Antigenicity, specificity, and immunogenicity structurally and functionally characterize antigens.

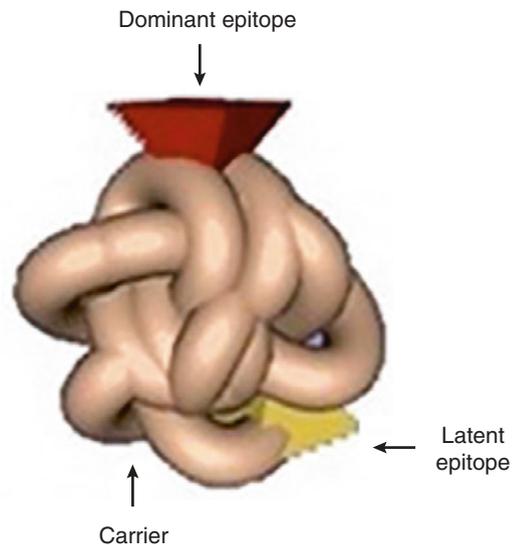
*Antigenicity* is the quality of an antigen to serve *ligand* for a *receptor*. The receptors for antigens are TCR and BCR.

*Specificity* is the antigen quality to be a unique molecule for only one receptor. An *epitope* or *antigenic determinant* is an informational unit of the antigen specificity. In the antigen molecule, an epitope may be dominant or latent (see ■ Fig. 1.1). A carrier, the noninformation part of the antigen molecule, is required for any antigen to be complete.

■ **Table 1.1** Antigens and haptens

Antigen type	Biochemical characterization of antigens	Immunogenicity (the power of immune response)	Formation of a precipitate with the specific antibody	Valence (a number of epitopes)
Complete antigens	Proteins, polysaccharides, lipopolysaccharides, and phospholipids	+	+	Polyvalent
Haptens (incomplete antigens):				
Complex haptens	Short peptides and saccharides, lipids, nucleic acids, and some medications	–	+	Divalent
Antigen type	Biochemical characterization of antigens	Immunogenicity (the power of immune response)	Formation of a precipitate with the specific antibody	Valence (a number of epitopes)
Simple haptens	Chemical radicals, amino acids, simple sugars, and other simple chemical substances	–	–	Monovalent

■ **Fig. 1.1** Antigen structure



*Immunogenicity* is the quality to induce the adaptive immune responses of different power.

The majority of antigens are *T dependent* since they require the participation of helper T cells to constitute memory cells. *T-independent* antigens are capable of activating B cells on their own. In the past, there was a division of T-independent antigens into two types: type 1 (currently they all refer to PAMPs – see below) and type 2, which comprised highly repetitive surface epitopes, e.g., polysaccharides of encapsulated bacteria. Type 2 T-independent antigens can interact with many BCRs in a cross-linking manner and activate only mature B cells, whereas immature B cells remain anergized.

**From a clinical point of view,** children up to 4–6 years who have most immature B cells cannot produce antibodies at the high level required for defense against encapsulated bacteria, which may often be reactivated.

Small exogenous molecules which contain a conserved motif, *pathogen-associated molecular patterns (PAMPs)*, are linked to a certain component of microbes. There are bacterial flagellin, peptidoglycan, lipopolysaccharide (endotoxin, LPS), viral dsRNA, and unmethylated CpG motifs of DNA. They may initiate different reactions of the *innate immunity* and do not induce immune memory. Other exogenous molecules, oligomeric components of allergen molecules, are *allergen-associated molecular patterns (AAMPs)*, which may promote cross-reactivity of IgE allergy. Some endogenous molecules, *damage-associated molecular patterns (DAMPs)*, are released outside the cell because of its injury. There are heat-shock proteins, extracellular matrix's (ECM's) proteins, S100, hyaluronan fragments, and nonprotein substances such as DNA, ATP, uric acid, and heparin. In physiological conditions, DAMPs serve structural and metabolic functions, being inaccessible to the immune system.

**From a clinical point of view,** in case of severe damage to own tissue, they may trigger peracute inflammation and toxification and promote toxic shock syndrome.

*Tumor-associated molecular patterns (TAMPs)* are low-molecular conserved components of tumor cells. On the one hand, they can upregulate innate defense against tumors, but, on the other hand, TAMP may, like a “double-edged sword,” promote cancer growth and metastasis through weakening immune surveillance, the formation of chemoresistance, and the chronic inflammation favoring tumor progression.

All the “patterns” evoke similar reactions of the innate immunity. They are recognized by Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs), which are currently an actively growing area of research.

## ■ Quiz

Reading a question, please choose only one right answer.

### ? Question 1

Antigens trigger:

1. NETosis.
2. Reactions of the innate immunity.
3. Adaptive immune responses.
4. Phagocytosis.

**? Question 2**

Complete antigens are:

1. Short peptides, and saccharides, lipids, nucleic acids.
2. Immunoglobulins.
3. Proteins, polysaccharides, lipopolysaccharides, and phospholipids.
4. Chemical radicals, amino acids, simple sugars.

**? Question 3**

Any epitope is:

1. An informational unit of the antigen specificity.
2. A pathogen-associated molecular pattern.
3. T-cell receptor.
4. B-cell receptor.

**? Question 4**

Tolerogen triggers:

1. Immune responses.
2. Innate immunity.
3. Phagocytosis.
4. Immune tolerance.

**? Question 5**

A complete antigen contains:

1. A carrier only.
2. Epitopes and a carrier.
3. Only antigenic determinants.
4. Damage-associated molecular patterns.

**? Question 6**

Haptens are:

1. Complete antigens.
2. A carrier for epitopes.
3. Incomplete antigens.
4. Pathogen-associated molecular patterns.

**? Question 7**

What TCR stands for?

1. T-cell receptor.
2. T cellular reaction.
3. T-cell-mediated response.
4. T-cell resistance.

**? Question 8**

Estimated number of “universe of antigens” is about:

1.  $10^{18}$ .
2.  $10^{13}$ .
3.  $10^{10}$ .
4.  $10^8$ .

**? Question 9**

Complex haptens are:

1. Polyvalent.
2. Ambivalent.
3. Monovalent.
4. Divalent.

**? Question 10**

B-cell receptor (BCR) can:

1. Trigger T-cell-mediated responses.
2. Recognize antigens.
3. Activate phagocytosis.
4. Activate complement.

**? Question 11**

Damage-associated molecular patterns (DAMPs) are:

1. Low-molecular conserved components of tumor cells.
2. Oligomeric components of allergens.
3. Bacterial flagellin, peptidoglycan, and lipopolysaccharide, viral dsRNA, etc.
4. Heat-shock proteins, ECM's proteins, S100, hyaluronan fragments, etc.

**? Question 12**

Pathogen-associated molecular patterns (PAMPs) are:

1. Complex haptens.
2. Low-molecular conserved components of tumor cells.
3. Bacterial flagellin, peptidoglycan, and lipopolysaccharide, viral dsRNA, etc.
4. Oligomeric components of allergens.

**? Question 13**

Allergen-associated molecular patterns (AAMPs) are:

1. Oligomeric components of allergens.
2. Heat-shock proteins.
3. Bacterial flagellin, peptidoglycan, and lipopolysaccharide.
4. Unmethylated CpG motifs of DNA.

**? Question 14**

All the "patterns" trigger:

1. Immune tolerance.
2. T lymphopoiesis.
3. Thymus involution.
4. Reactions of the innate immunity.

**? Question 15**

Toll-like receptors (TLRs) are related to:

1. Hormone receptors.
2. Pattern recognition receptors (PRRs).
3. Cytokine receptors.
4. Chemokine receptors.

**? Question 16**

Antigens may be derived from:

1. "Former self" only.
2. "Non-self" only.
3. "Non-self," "former self," and "self."
4. "Self" only.

## 1.3 Immunological Mechanisms

---

### Definitions

*Immunity* (Latin "immunitas") is a universal biological phenomenon that develops many programs based on the unique genotype of the body ("self") in foreign surroundings, from the birth of the body to its death. There are two major types of immunity, *innate immunity*, which is phylogenetic and polyspecific, and *adaptive immunity*, which is acquired during an ongoing individual life.

*Immunology* is a life science that studies the immune system, immunological mechanisms, and immunopathology in humans, animals, and other living beings.

In contrast to other systems, the *immune system* is responsible for support of the balance or homeostasis between "non-self," "self," and "former self." The end effects of two major types of immunological mechanisms, innate immunity and adaptive immunity, may be:

1. *Immune containment* of infection and tumors
2. *Immune clearance* of the infection and tumors.

### 1.3.1 Innate Immunity

---

The innate immune subsystem upon activation has a wide array of recruited molecules and cells, which may destroy invading pathogens very quickly but not very effectively.

1. *Physical and chemical barriers*:
  - Keratinization in the skin
  - Mucus formation on the mucosal epithelium and ciliary clearance in the respiratory tract
  - Production of various antimicrobial factors such as lysozyme, lactic and fatty acids, etc. in secretions
  - Deactivation of dangerous microbes by digestive enzymes and peristalsis in the GI tract
2. *Microbial antagonism* to pathogenic microbes due to the body's own mutualistic and commensal microorganisms
3. The *liver* due to oxidation of xenobiotics, detoxification, and synthesis of many defense factors
4. *Cytotoxicity by complement*
5. *Phagocytosis* and *NETosis*

6. *Acute phase reaction* (C-reactive protein, serum amyloid A, mannose-binding lectin, etc.)
7. *Natural antibodies* produced by CD5+B cells
8. *Antimicrobial peptides* such as  $\alpha$  defensins, cathelicidins, lactoferrin, dermicidin, etc.
9. *Natural cytotoxicity* due to innate lymphoid cells (ILCs) including NK cells, NKT cells, and  $\gamma\delta$ T cells plus *natural cytostasis* induced by interferons (IFNs)

### 1.3.2 Adaptive Immunity

---

The adaptive immune responses take some days and weeks to be finished. However, they are more effective in eliminating invading pathogens than the innate immunity. Furthermore, they develop the immune memory to the invading pathogens.

#### ■ B-Cell-Mediated (Humoral) Responses

1. *Simple B-cell response* – formation of only one class of immunoglobulins, IgM, but no long-term memory. This type of response may be triggered by “patterns” too.
2. *Advanced B-cell response* – switching antibodies after each other: IgM, IgG, IgA, and even IgE, and inducing the formation of long-lived memory plasma cells and lifelong memory B cells.

#### ■ T-Cell-Mediated Responses

3. *Inflammatory CD4+T-cell response* that leads to the production of effector CD4+T cells and the lifelong memory CD4+T cells.
4. *Cytotoxic CD8+T-cell response*, which results in the formation of cytotoxic CD8+T cells capable of apoptosis in target cells and lifelong memory CD8+T cells.

## 1.4 Organization of the Immune System at a Glance

---

The immune system of the body consists of organs, cells, and molecules, and a complex interplay of them all governs immune processes at two major levels. They may be defined as the following:

1. *Systemic level*, which includes the bloodstream, thymus, bone marrow, and spleen and is responsible for defense against pathogens if they invade the internal space of the body
2. *Skin and mucosal level* that includes the surface and mucosal barriers, tonsils, adenoids, Peyer’s patches, solitary or isolated follicles, appendix, lymph nodes, lymphatic vessels, etc. at which the immune system functions if the pathogens enter the body locally or the barrier’s opportunistic microbes are being reactivated

The primary or central organs of the immune system are the *thymus* and *bone marrow*. All cells related to the immune system originate from the bone marrow, and even some lymphocytes, B cells, are differentiated there, whereas other lymphocytes, T cells, are matured in the thymus. Furthermore, the thymus governs the whole immune system.

The secondary or peripheral organs of the immune system include the *spleen, lymph nodes, numerous disseminated lymphoid elements, appendix, lymphatics, skin, and even liver*. An essential part of the secondary organs is organized in *mucosae-associated lymphoid tissue (MALT)*, which may become a place where many immune processes of the innate and adaptive immunity proceed to protect the body against numerous pathogens.

If the participation in the immune processes to take into consideration cells of the immune system may be classified by their functional activity and divided into four groups:

1. Antigen-Presenting Cells (APCs):  
Dendritic cells (DCs), macrophages, and B cells
2. Immunoregulatory cells:  
Natural T regulatory cells (nTreg) and their induced subsets, natural B regulatory cells (nBreg)  
Adaptive helper T-cell subsets: type 1 helper T cells (Th1), type 2 helper T cells (Th2), follicular helper T cells (Tfh), follicular regulatory T cells (Tfr), type 9 helper T cells (Th9), type 17 helper T cells (Th17), and type 22 helper T cells (Th22)
3. Effector cells:  
Inflammatory CD4+T cells, cytotoxic CD8+T cells, plasma cells as antibody-producing cells,  $\gamma\delta$ T cells, NKT cells, innate lymphoid cells (ILCs) including NK cells, monocytes, macrophages, neutrophils, eosinophils, mast cells, etc.
4. Memory cells:  
Memory CD4+T cells, memory CD8+T cells, memory B cells, long-lived plasma cells

Molecules of the immune system may be divided into some groups:

1. Antigen-recognizing and antigen-binding molecules:
  - Immunoglobulins or antibodies: IgM, IgG, IgA, IgE, and IgD
  - B-cell receptor (BCR)
  - T-cell receptor (TCR)
  - Transfer factors (soluble TCR's fragments)
  - Human histocompatibility antigens (HLA)
2. Pattern recognition receptors (PRRs):
  - Toll-like receptors (TLRs)
  - C-type lectin receptors (CLRs)
  - NOD-like receptors (NLRs)
  - RIG-1-like receptors (RLRs)
  - AIM-2-like receptors (ALRs)

3. Cell adhesion molecules (CAMs):
  - Immunoglobulin superfamily
  - Integrins
  - Selectins
  - Mucosal vascular addressins or mucin-type glycoproteins
  - Tumor necrosis factor (TNF) receptor superfamily
  - Cadherin superfamily
  - Extracellular matrix (ECM) proteins or Link family
4. Cytokines and chemokines:
  - Interleukins (ILs)
  - Colony-stimulating factors (CSFs)
  - Interferons (IFNs)
  - Tumor necrosis factors (TNFs)
  - Chemokines
  - Others
5. Various mediators of immune inflammation

### Definitions

A *ligand* is a soluble molecule, which is bound to a complementary or specific *receptor* expressed on a cell. The receptor may also be bound to a *counter-receptor* on another cell.

*Signaling* is a series of reactions from the ligand/receptor complex toward the genome of the target cell, which results in a particular action or functional activity of the cell.

**CD Nomenclature.** *CD* means *cluster of differentiation*. Due to the hybridoma technology developed by the Nobel Laureates G.J.F. Köhler and C. Milstein (1984), it has become possible to define certain molecules, which are expressed at the different stages of cell differentiation. *CD* molecules may be signaling molecules, receptors, counter-receptors, cell adhesion molecules, etc. The use of the monoclonal antibodies enabled the identification of cell surface molecules providing targets for immunophenotyping of cells. Currently, it is a conventional rule to utilize them as cell markers in immunology (see ■ Table 1.2). These markers are also applied to link cells of the immune system with certain immune functions. To date, the *CD* molecules for humans are numbered up to 371.

To summarize the general vision of the immune system at a glance, see ■ Table 1.3.

■ **Table 1.2** Some CD markers

Cluster designation	Cells
CD34+	Lymphoid and myeloid progenitors
CD3+	T cell
CD4+	Helper T cell
CD8+	Cytotoxic T cell
CD19+	B cell
CD16 <sup>hi</sup> 56 <sup>lo</sup>	NK cell
CD68+	Macrophage
CDw199+	CC chemokine receptor type 9, encoded by CCR9 gene; a $\beta$ chemokine receptor involved in mucosal immunity

*hi* denotes *high* expression, *lo* means *low* expression, *w* means *workshop* (not well-characterized to use this molecule as a conventional marker)

■ **Table 1.3** “Formula” of immunity

Feature	Innate immunity	Adaptive immunity
Trigger	“Patterns”	Antigens
Development	Rapid	Slow
The fate of a pathogen	Immune containment	Immune clearance
Memory	Phylogenetic polyspecific memory to pathogens; no formation of monoclonal memory after a primary infection	Formation of long-term monoclonal memory after a primary infection
Crucial cells	Phagocytes, NK cells, mast cells, etc.	T cells and B cells
Effector events	“Acute phase” reaction, complement activation, phagocytosis, NETosis, pyroptosis, simple inflammation, apoptosis	Antigen neutralization by antibodies, CD4+T-cell-initiated immune inflammation, CD8+T-cell-induced apoptosis in target cells
Paradigm	Pattern recognition theory	Clonal selection theory
Immunopathology	Immunodeficiency, autoinflammatory disorders	Immunodeficiency, autoimmune diseases, allergic disorders

## 1

## ■ Quiz

Reading a question, please choose only one right answer.

## ? Question 1

Systemic level's organs are:

1. Mucosal barriers.
2. Thymus, bone marrow, and spleen.
3. Peyer's patches and isolated follicles.
4. Lymph nodes and lymphatic vessels.

## ? Question 2

The primary organs of the immune system are:

1. The skin.
2. Mucosal barriers, tonsils, and adenoids.
3. Thymus and bone marrow.
4. Peyer's patches, isolated follicles, and appendix.

## ? Question 3

The skin and mucosal level includes:

1. Mucosal barriers, tonsils, and adenoids.
2. The thymus, bone marrow, and spleen.
3. The central nervous system.
4. Endocrine glands.

## ? Question 4

Adaptive immunity is:

1. Immune responses.
2. Reactions of innate immunity.
3. Phagocytosis.
4. Complement.

## ? Question 5

Pathogen-associated molecular patterns (PAMPs) are:

1. Complex haptens.
2. Low-molecular conserved components of tumor cells.
3. Bacterial flagellin, peptidoglycan, and lipopolysaccharide, viral dsRNA, etc.
4. Oligomeric components of allergens.

## ? Question 6

Antigen-presenting cells (APCs) are:

1. T cells.
2. Natural T regulatory cells (nTreg).
3. Dendritic cells (DCs), macrophages, and B cells.
4. NK cells.

**? Question 7**

The clonal selection theory explains:

1. Adaptive immunity.
2. Formation of inflammasomes.
3. NETosis.
4. Innate immunity.

**? Question 8**

What BCR stands for?

1. B-cell receptor.
2. B cellular reaction.
3. B-cell-mediated response.
4. B-cell resistance.

**? Question 9**

Neutrophils are related to:

1. Antigen-presenting cells (APCs).
2. Memory cells.
3. Follicular regulatory T (Tfr) cells.
4. Effector cells.

**? Question 10**

Selectins are:

1. Immunoglobulins.
2. Cell adhesion molecules (CAM).
3. Cytokines.
4. Antigen-recognizing and antigen-binding molecules.

**? Question 11**

T-cell receptor (TCR) can:

1. Trigger B-cell-mediated responses.
2. Recognize antigens.
3. Activate phagocytosis.
4. Activate complement.

**? Question 12**

Damage-associated molecular patterns (DAMPs) are:

1. Low-molecular conserved components of tumor cells.
2. Oligomeric components of allergens.
3. Heat-shock proteins, ECM's proteins, S100, hyaluronan fragments, etc.
4. Bacterial flagellin, peptidoglycan, and lipopolysaccharide, viral dsRNA, etc.

**? Question 13**

Interleukins (ILs) are related to:

1. Cytokines.
2. Cell adhesion molecules (CAM).
3. Pattern recognition receptors (PRRs).
4. Human histocompatibility antigens (HLA).

**? Question 14**

All the “patterns” trigger:

1. B cells.
2. T cells.
3. Thymus involution.
4. Reactions of the innate immunity.

**? Question 15**

CD3 molecules are expressed by:

1. B cells.
2. T cells.
3. Macrophages.
4. Eosinophils.

**? Question 16**

Antigens may be derived from:

1. “Former self” only.
2. “Non-self” only.
3. “Self” only.
4. “Non-self,” “former self,” and “self.”

## 1.5 Molecules of the Immune System

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Methodically, molecules of the immune system will be presented in detail before organs and cells of the system are considered.

### 1.5.1 Antigen-Recognizing and Antigen-Binding Molecules

---

#### Definitions

*Immunoglobulin* or *antibody* is an effector molecule of the B-cell-mediated responses, which is secreted by plasma cells and interacts to appropriate antigen specific to this antibody. In *Homo sapiens*, there are *IgM*, *IgG*, *IgA*, *IgE*, and *IgD* classes of the immunoglobulins.

*Antigen-recognizing receptors* themselves sense antigens. However, each antigen-recognizing complex of B cells and T cells consists of:

1. An *antigen-recognizing receptor* itself, which recognizes “non-self” and “former self”
2. An *accessory antigen receptor’s molecule*, which is required for signaling and re-expressing antigen-recognizing receptors
3. A *coreceptor*, which recognizes HLA molecules (“self”)

*Human leukocyte antigen (HLA) molecule* is a major histocompatibility complex in *Homo sapiens*. *Class I HLA* molecules are expressed on cell surfaces throughout the body, whereas *Class II HLA* molecules are displayed on cells of the immune system only.

**■ Quiz**

Reading a question, please choose only one right answer.

**? Question 1**

The coreceptor, which senses HLA II molecules, is:

1. CD8.
2. CD16.
3. CD4.
4. CD22.

**? Question 2**

What the immunoglobulin molecule is pentameric?

1. IgM.
2. IgG.
3. IgA.
4. IgE.

**? Question 3**

These molecules inform cells of the immune system about the autologous state of cells on which they are expressed:

1. LFA-1.
2. CD3.
3. CD4.
4. HLA I.

**? Question 4**

HLA genes are located on chromosome:

1. 6.
2. 14.
3. 7.
4. 22.

**? Question 5**

A groove in the HLA molecule is required:

1. For HLA expression.
2. For antigen loading.
3. For HLA splitting.
4. For HLA polymorphism.

**? Question 6**

This immunoglobulin is divided into subclasses:

1. IgM.
2. IgD.
3. IgG.
4. IgE.

**? Question 7**

This immunoglobulin exerts a quality to placental transfer:

1. IgM.
2. IgE.
3. IgG.
4. IgA.

**? Question 8**

Molecule, non coreceptor, associated to TCR is:

1. CD3.
2. CD4.
3. CD8.
4. CD79a/CD79b.

**? Question 9**

Molecules are associated to BCR are:

1. CD3 chains.
2. CD79a/CD79b.
3. CD4 and CD8.
4. Cytokines.

**? Question 10**

IgG is synthesized at low concentration:

1. In senescence.
2. In babies at the age of 3–6 months.
3. In teenagers.
4. In pregnant women.

**? Question 11**

By which part does an immunoglobulin bound an antigen?

1. By Fc fragment.
2. By hinge area.
3. By Fab fragment.
4. By constant domains.

**? Question 12**

Immunoglobulins are synthesized by:

1. Plasma cells.
2. T cells.
3. Mast cells.
4. Macrophages.

**? Question 13**

The coreceptor, which recognizes HLA I molecules, is:

1. CD4.
2. CD21.
3. CD8.
4. CD19.

**? Question 14**

Diversity of antibodies inside a species is:

1. Allotypy.
2. Isotypy.
3. Affinity.
4. Idiotypy.

**? Question 15**

IgE is responsible for:

1. Type I hypersensitivity.
2. Type II hypersensitivity.
3. Type III hypersensitivity.
4. Type IV hypersensitivity.

**? Question 16**

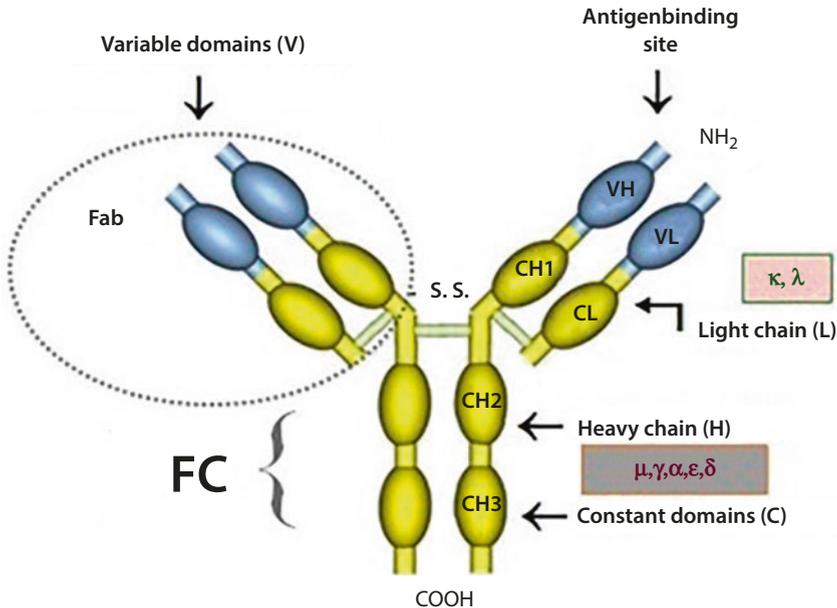
Antibody, which indicates either any recent pathogenic infection or a reactivation of opportunistic microbes:

1. IgA.
2. IgE.
3. IgG.
4. IgM.

### 1.5.1.1 Immunoglobulins

*Immunoglobulins* or *antibodies* are effector molecules of the B-cell-mediated responses, which are secreted by plasma cells. Any antibody is a glycoprotein composed of many amino acid residues, and that is its primary structure. The carbohydrate content of the immunoglobulin molecules varies between 2% and 12%. A monomeric IgG molecule consists of two identical *light* (*L*) and two identical *heavy* (*H*) chains (see ■ Fig. 1.2). They are attached to each other by disulfide (S-S) and hydrogen (H+...O-) bonds forming the secondary structure of the immunoglobulin molecule. Both the chains contain a series of repeating, homologous units, which fold in separation into a domain in a globular manner, and that is the tertiary structure of the immunoglobulin molecule. In addition, there are *constant* (*CL*, *CH1*, *CH2*, and *CH3*) and *variable* (*VL* and *VH*) domains. The molecule can be divided into Fc fragment (“fragment crystallizable”), responsible for nonspecific effector activity, and two identical Fab fragments or antigen-binding sites, which bind antigens. Finally, the whole molecule is conformed as the functionally active compound. That is the quaternary structure of the immunoglobulin molecule.

The cleavage of a monomeric antibody with papain enables the obtaining of two different fragments, two Fab and one Fc. Each Fab fragment can bind a single antigen molecule with no precipitation, whereas an Fc fragment can constitute crystals. The cleavage of a monomeric antibody with pepsin enables the obtaining of one fragment composed of two Fc fragments and capable of binding two antigen molecules with precipitation. Finally, the cleavage of a monomeric antibody with disulfide enables the obtaining of separated H chains and L chains.



■ Fig. 1.2 Structure of the immunoglobulin molecule

Antibodies are characterized by some qualities as follows.

- *Affinity* is the quality of an immunoglobulin molecule to be bound to antigen, one to one, firmly on the base of close agreement of their specificities.
- *Avidity* is the same quality based on polyvalence of the antigen-binding sites.
- *Cross-reactivity* is the ability of one antibody to bind to different antigenic epitopes.
- *Isotypy* is a diversity of antibodies inside a species. In humans, there are IgM, IgD, IgG, IgA, and IgE isotypes or classes.
- *Allotypy* is an individual antibody diversity based on the inheritance of different alleles.
- *Idiotypy* is a clonal antibody diversity.

Antibody isotypes differ from each other in some qualities (see ■ Table 1.4).

The Nobel Prize in 1972 was awarded jointly to G.M. Edelman and R.R. Porter for their research on the chemical structure of antibodies.

Genes for different chains are located on different chromosomes. Genes on chromosome 2 encode  $\kappa$ -type L chains, genes on chromosome 22 are related to  $\lambda$ -type L chains, and genes on chromosome 14 encode H chains.

*IgM* is the sizeable pentameric antibody secreted into the blood during a simple B-cell-mediated response and at the beginning of an advanced B-cell-mediated response. As a rule, *IgM* has low affinity and high avidity for the antigen.

**From a clinical viewpoint,** *IgM* may indicate either any recent pathogenic infection or a reactivation of opportunistic microbes. *IgM* can mobilize the 1st component of complement and opsonize bacteria and fungi during phagocytosis. In healthy adults, it makes up 0.6–2.0 g/L. As a monomer, *IgM* is a part of BCR on immature B cells.

■ **Table 1.4** Physicochemical qualities of immunoglobulin isotypes

Feature	IgM	IgG	IgA	IgE	IgD
Form	Pentameric	Monomeric	Monomeric (serum IgA) or dimeric (secretory IgA)	Monomeric	Monomeric
Heavy chain	$\mu$	$\gamma$	$\alpha$	$\epsilon$	$\delta$
Accessory chain	J		Secretory component (SC) including pIgR		
Subisotypes (subclasses)		1 (65%) 2 (20%) 3 (10%) 4 (5%)	1 2		
Number of constant domains	40H, 10 L	6H, 2 L	6H, 2 L (12H, 4 L)	8H, 2 L	6H, 2 L
Molecular mass (kDa)	950	150	160 (385)	190	180
Half-life (days)	5	23	6	2.5	3
Serum concentration in healthy adults (g/L)	0.6–2.0	8–16	0.7–3.0	0.003	0.04
Placental transfer		+			
Fc receptors		Fc $\gamma$ R I (CD64) Fc $\gamma$ R II (CD32) Fc $\gamma$ R III (CD16)	Fc $\alpha$ R (CD89)	Fc $\epsilon$ R I <sup>hi</sup> Fc $\epsilon$ R II <sup>lo</sup> (CD23)	

*IgD* is another part of the BCR, which appears on mature B cells. Soluble *IgD* is present in the bloodstream in a small concentration but may participate in some immune processes such as defense, tolerance, and allergic reactions. Also, *IgD* is expressed by anergic B cells.

*IgG* is the most common isotype of antibody found in the bloodstream. In healthy adult humans, *IgG* accounts for 6.0–16.0 g/L. *IgG* is characterized by high affinity for antigen having only two antigen-binding sites though. There are four *IgG* subisotypes (*IgG1*, *IgG2*, *IgG3*, and *IgG4*) in humans, named in order of their abundance in the bloodstream. The immunoglobulins of almost all subisotypes provide the most effective

humoral defense against extracellularly located pathogens. IgG antibodies are generated following isotype switching and maturation of plasma cells. IgG molecules may neutralize pathogens by agglutination, coat them in an opsonization way facilitating phagocytosis, upregulate the complement via a classical pathway analogous to IgM, and take part in antibody-dependent cell-mediated cytotoxicity (ADCC). Unfortunately, some IgG subisotypes may be associated with type I, type II, and type III allergic reactions. For example, IgG4 is linked to type I hypersensitivity analogous to IgE.

**From a clinical point of view,** a decrease in IgG synthesis based on an inherited inability to produce IgG is often a severe medical problem because this form of *primary antibody immunodeficiency* is accompanied by hard recurrent pyogenic infections (see ► Sect. 6.2) that requires lifelong intravenous immunoglobulin (IVIG) administration.

There is a characteristic feature of IgG synthesis in babies. At birth, a baby has 100% of maternal IgG. However, the maternal antibodies degrade between the 3rd and 5th months during a period called *physiological hypogammaglobulinemia*. Meanwhile, by the 6th month, most babies have already synthesized about 1/3 of their self IgG, and then they progressively save more.

IgA has two forms (monomeric and dimeric) and two subisotypes (IgA1 and IgA2). IgA1 is often produced as a monomeric antibody and predominates in the bloodstream, whereas IgA2, a preferentially dimeric form, is prevalent in secretions and is also called secretory IgA (sIgA). In healthy adults, serum IgA accounts 0.7–3.0 g/L, and sIgA varies from one secretion to another. The major quality of both IgA and sIgA is the ability to neutralize microbial toxins. Each sIgA contains a particular polypeptide, secretory component (SC). The SC includes a polymeric Ig receptor (pIgR), which is responsible for the transport of IgA across the mucous membrane epithelial cells and into secretions such as breast milk, saliva, gut fluid, sperm, etc. Breastfeeding is extremely important for development, growth, and defense of any baby due to sIgA and other immune components of the breast milk.

**From a clinical point of view,** IgA A decrease in IgA synthesis based on an inherited inability to secrete IgA is termed *selective IgA deficiency*. The mutations are located in the IGAD1 locus on 6p21 and IGAD2 locus on 17p11. This benign form of primary immunodeficiency may be revealed in patients very often (about 1% of the population) but may or not have any essential clinical significance. However, IgA deficiency is occasionally linked with autoimmune pathology.

IgE is produced in response to allergens. It is a rare antibody in the bloodstream but located in many tissues due to ligation with  $Fc_{\epsilon}RI$  and  $Fc_{\epsilon}RII$  on some cells. IgE is the only cytophilic antibody among other immunoglobulins. The high-affinity  $Fc_{\epsilon}RI$  is expressed on basophils and mast cells and binds to IgE/allergen complex that leads to *type I hypersensitivity* in allergic patients with asthma, perennial and/or seasonal rhinitis, atopic dermatitis, etc. Upon degranulation, being a mediator of allergic inflammation at the early phase reaction, histamine is capable of engaging many cell types to forward inflammatory process to the late-phase reaction that turns the early allergic episode into chronic allergic disease. IgE antibodies are unique to each allergen. For example, IgE secreted in response to a house dust mite differs from IgE produced after a wasp sting.

**From a clinical viewpoint,** for examination of *type I* or *IgE-linked hypersensitivity*, *allergic skin tests* are more appropriate and reliable as opposed to *allergen-specific IgE blood tests* because IgE antibodies have the cytophilic quality to be linked in tissues (e.g., the mucosae and skin).

### 1.5.1.2 Fc Receptors

Fc receptors (FcRs) for immunoglobulins can connect antibodies with cells. Currently, Fc receptors for all isotypes of antibodies have been identified. There are Fc $\mu$ R (for IgM), Fc $\gamma$ R (for IgG), Fc $\alpha$ RI (for IgA), Fc $\epsilon$ RI (for IgE), and Fc $\delta$ R (IgD). For IgG, three classes of Fc receptors are found: CD64 (Fc $\gamma$ RI), CD32 (Fc $\gamma$ RIIa, Fc $\gamma$ RIIb, and Fc $\gamma$ RIIc), and CD16 (Fc $\gamma$ RIIIa and Fc $\gamma$ RIIIb). Fc $\gamma$ RI is a high-affinity Fc receptor, whereas Fc $\gamma$ RII and Fc $\gamma$ RIII have a lower affinity.

### 1.5.1.3 B-cell Receptors (BCR)

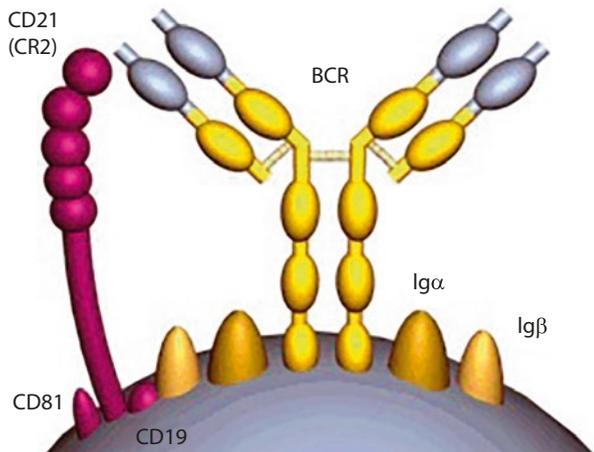
An antigen-recognizing B-cell receptor (BCR) consists of monomeric IgM and IgD (see ■ Fig. 1.3) and capable of recognizing and binding antigens (“non-self”). BCRs are characterized by cross-linking to antigens.

BCR is associated with two specialized accessory molecules, Ig $\alpha$  (CD79a+) and Ig $\beta$  (CD79b+), which are required for signaling and re-expressing BCR. In addition, there is the coreceptor, CD19+/CD21+/CD81+ that probably binds antigen/Class II HLA molecules during the “dual recognition,” i.e., the recognition of “non-self” and “self.” CD22+ serves as an inhibitory coreceptor.

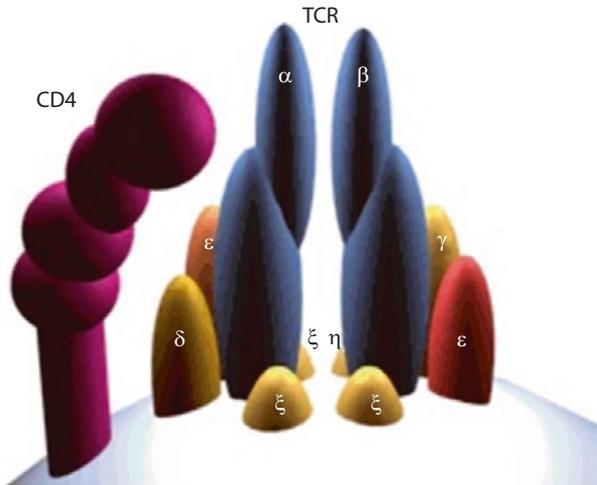
### 1.5.1.4 T-Cell Receptors (TCR)

An antigen-recognizing T-cell receptor (TCR) may be composed of  $\alpha$  chain and  $\beta$  chain (type  $\alpha\beta$ TCR) and  $\gamma$  chain and  $\delta$  chain (type  $\gamma\delta$ TCR).  $\alpha\beta$ TCR T cells originate from the thymus, whereas  $\gamma\delta$ TCR T cells ( $\gamma\delta$ T cells) are differentiated in the thymus and outside the thymus. Each chain has a variable and a constant domain (see ■ Figs. 1.4 and 1.5).  $\alpha\beta$ TCR reacts only to peptide antigens, whereas  $\gamma\delta$ TCR can react to phospholipid antigens (phosphoantigens) probably presented by CD1.

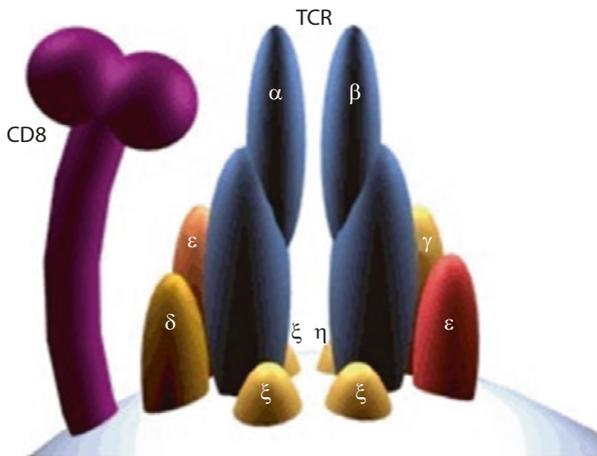
■ Fig. 1.3 B-cell receptor (BCR)



■ Fig. 1.4 T-cell receptor (TCR) CD4+



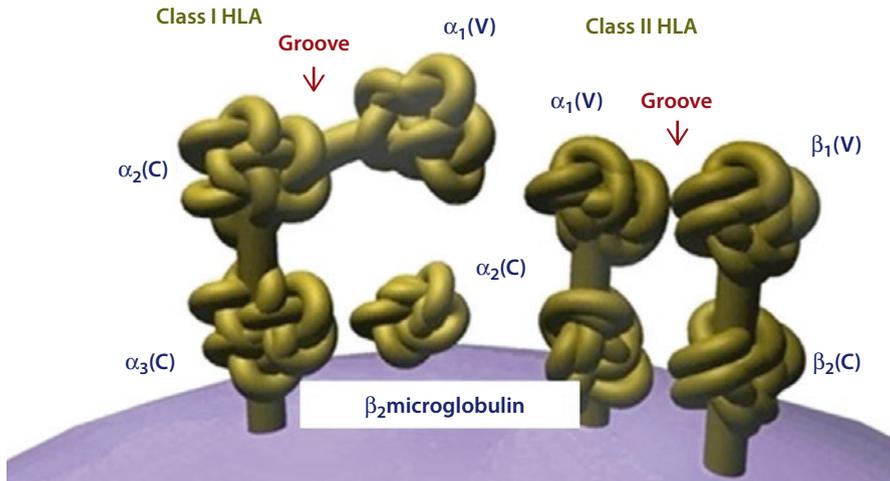
■ Fig. 1.5 T-cell receptor (TCR) CD8+



CD3, an accessory molecule, is associated with TCR and required for signaling and re-expressing TCR. The CD3 consists of four dimeric parts,  $\epsilon\delta$ ,  $\epsilon\gamma$ ,  $\xi\xi$ , and  $\xi\eta$ . Two types of T-cell coreceptors differ from each other in the ability to recognize either Class I HLA molecules (CD8+ T cells) or Class II HLA molecules (CD4+ T cells). Analogous to B cells, T cells also take part in “dual recognition” using TCR for antigens (“non-self”), CD4 for Class II HLA (“self”), and CD8 for Class I HLA (“self”). Depending on the presence of either the CD4 coreceptor or the CD8 coreceptor, all the T cells are divided into two major subpopulations, helper/inflammatory CD4+ T lymphocytes and cytotoxic CD8+ T lymphocytes.

Interestingly, about 30% of T cells may simultaneously express with two TCRs of different specificities.

**From a clinical point of view,** there are many mutations in genes encoding the different chains of BCR and TCR that leads to *primary immunodeficiencies* (see ► Sect. 6.2).



■ Fig. 1.6 Human leukocyte antigen (HLA) molecules

### 1.5.1.5 Human Leukocyte Antigens (HLA)

A major histocompatibility complex in humans is presented by human leukocyte antigens (HLA). A *Class I HLA* molecule consists of two chains,  $\alpha$  (three domains) and  $\beta_2$  microglobulin (B2B) (see ■ Fig. 1.6), and a *Class II HLA* molecule is also composed of two chains,  $\alpha$  and  $\beta$ . There are variable and constant domains depending on non-conserved and conserved sequences of amino acids. *Peptide-binding grooves* are located between two domains of both Class I and Class II HLA molecules to upload antigenic epitope during antigen processing. Specialized molecules, *chaperones* like Ii chain, HLA-DM, and HLA-DO for Class II HLA molecules and calnexin, calreticulin, and tapasin for Class I HLA molecules, are responsible for folding/unfolding and assembly/disassembly of the whole HLA/antigen complex. The molecular chaperones are unable to bind antigenic peptides themselves, but they “maintain” the grooves before antigen upload and regulate all stages of antigen processing and the HLA/antigen complex presentation to lymphocytes.

B. Benacerraf, J. Dausset, and G.D. Snell won the Nobel Prize in 1980 for their discoveries concerning genetically determined structures on the cell surface (HLA) that regulate immunological reactions. HLA genes are located on chromosome 6, within the 6p21.3 region.

HLA molecules play many roles in immune processes:

1. Autologous cells inform each other using expression of identical Class I HLA molecules on cell surfaces throughout the body.
2. Class I HLA molecules provide each person with individual superficial polymorphism.
3. Both Class I and Class II HLA molecules are required for antigen processing and uploading and presentation to lymphocytes so that the immune responses may progress.
4. Both Class I and Class II HLA molecules are responsible for the power of immune responses.

1

From a clinical viewpoint, HLA typing is especially essential during preparation for transplantation of any organ, disputed paternity and person's identification (including forensic use), scientific research of ancient sources, etc.

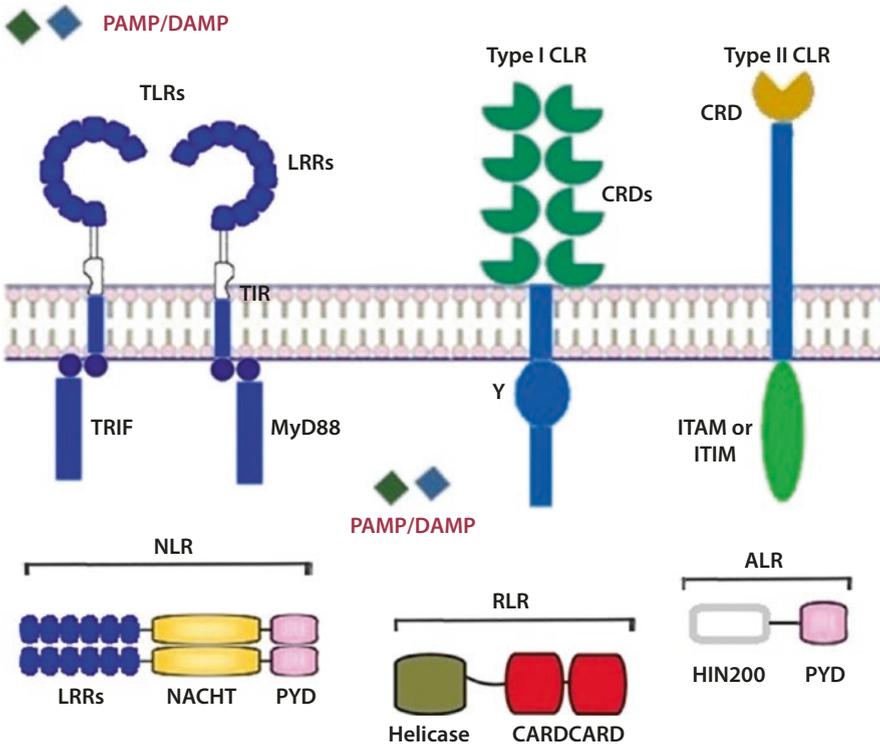
### 1.5.2 Pattern Recognition Receptors (PRRs)

**Definitions**

*Pattern recognition receptors (PRRs)* are molecules expressed by cells of the innate immunity, which are capable of sensing “patterns,” triggering the reactions of innate immunity such as inflammation and taking part in adaptive immune responses.

Typically, pattern recognition receptors (PRRs) contain one or several C-terminal recognizing or regulatory domains, which sense “patterns,” and N-terminal effector domains, which are associated with signaling molecules. Some PRRs have a central domain. To date, five families of PRRs have been described, as follows (see

■ Fig. 1.7):



■ Fig. 1.7 Pattern recognition receptors (PRRs)

■ **Table 1.5** TLRs and their ligands

TLR	Ligands (PAMP, DAMP)
TLR1	Triacyl lipopeptides of <i>Mycobacterium tuberculosis</i> , DAMP
TLR2	Diacyl and triacyl lipopeptides of Gram-positive bacteria, lipoteichoic acid, peptidoglycan, yeast zymozan, DAMP
TLR3	Viral dsRNA
TLR4	Lipopolysaccharide (endotoxin, LPS) of Gram-negative bacteria, DAMP
TLR5	Flagellin of bacterial flagella
TLR6	Diacyl lipopeptides of Gram-positive bacteria
TLR7	Viral ssRNA
TLR8	Viral ssRNA
TLR9	Unmethylated CpG nucleotides of bacterial and viral DNA
TLR10	Unknown

1. Toll-like receptors (TLRs)
2. C-type lectin receptors (CLRs)
3. NOD-like receptors (NLRs)
4. RIG-1-like receptors (RLRs)
5. AIM-2-like receptors (ALRs)

*Toll-like receptors (TLRs)* are expressed on the (i) membranes of macrophages, neutrophils, dendritic cells, lymphocytes, epitheliocytes, platelets, splenocytes, atherosclerotic plaques, etc. and present in the (ii) endosomes of the cells. The term “toll” comes from *Drosophila*, in which TLRs were first identified. TLRs contain leucine-rich repeat (LRR) domains and a Toll/interleukin1 receptor (TIR) domain, which is linked to signaling molecules including MyD88, TRIF, etc.

After binding to ligands (see ■ Table 1.5), most TLRs transduce signals in MyD88-dependent and TRIF-dependent pathways and eventually lead to the activation of inflammasome and pyroptosis, a base of the physiological inflammation. TLRs can recognize both PAMPs and DAMPs and provide a link between innate and adaptive immunity as they can interact with antigens too. A pathogen bound to TLRs may be engulfed, digested, and presented to lymphocytes in antigenic form.

*C-type lectin receptors (CLRs)* are categorized by the structure in type I and type II and by recognition motif in groups I and II (see ■ Table 1.6). They all are bound to the cell membranes and mainly involved in fungal recognition and endocytosis. Analogous to TLR, CLRs may recognize both “patterns” and antigens being a bridge between innate and adaptive immunity.

Type I CLRs consist of several carbohydrate recognition domains (CRDs) linked to cytoplasmic tails with a Y motif for signaling. Type II CLRs contain only one CRD, and their cytoplasmic tail includes either ITIM (immunoreceptor tyrosine-based inhibitory

**Table 1.6** C-type lectin receptors (CLR)

CLR	Recognition motif	PAMP, DAMP
Group I CLRs – The mannose receptors subfamily	Mannose	Viruses, fungi, mycobacteria
	Fucose	Bacteria, helminths
	Glucans	Fungi, mycobacteria
Group II CLRs – The asialoglyco-protein receptor subfamily	Galactose, proteins	Many PAMP and DAMP

motif), or ITAM (immunoreceptor tyrosine-based activating motif), or other sequence motifs to be a start point of signaling pathways. The members of type II CLRs are predominant in comparison with type I CLRs and are at the cutting edge of current research. They play many roles in antifungal, antimycobacterial, antitumor immunity, allergy, and maintenance of tissue homeostasis. For example, the study of Dectin-1 has revolutionized our understanding of human body-fungal interactions, whereas research on Dectin-2 has discovered that some CLRs may sense allergens such as house dust mite allergens as well as allergen-associated molecular patterns (AAMP).

Soluble type CLRs are related to “acute phase” proteins (see ► Chap. 3).

*NOD-like receptors (NLRs)* are localized in the cytosol. The term “NOD” comes from a nucleotide-binding oligomerization domain, which binds nucleoside triphosphate. NOD1 receptors recognize peptidoglycan only of Gram-negative bacteria, whereas NOD2 molecules recognize intracellular muramyl dipeptide, peptidoglycan of both Gram-positive and Gram-negative bacteria. NLRs commonly consist of LRR domains, a central NACHT domain and an effector domain, which may be one of five types, including PYD domain (*pyrin domain*). The term “NACHT” comes from *NAIP*, an apoptosis inhibitor protein; *C2TA*, a Class II HLA transcription activator; *HET-E*, an incompatibility locus protein from *Podospora anserina*; and *TPI1*, a telomerase-associated protein. A NACHT domain consists of 7 different conserved motifs, contains 300–400 amino acids, and exerts NTPase activity. A PYD domain takes part in the formation of an inflammasome, responsible for activating caspase-1, pro-inflammatory cytokines such as IL1 $\beta$  and IL18, pyroptosis, and starting the inflammatory process (see ► Chap. 3).

*RIG-1-like receptors (RLRs)* are present in the cytoplasm and consist of an RNA helicase domain and CARDS (caspase activation and recruitment domains). A ligand for RLRs is a viral PAMP, dsRNA. Activation of RLRs leads to type I interferon production.

*AIM-2-like receptors (ALRs)* are located in the cytoplasm and nucleus and contain a certain number of HIN200 domains and PYD domain. The term “HIN200” comes from “hematopoietic expression, interferon-inducible nature, and nuclear localization of 200 amino acids.” A ligand for ALRs is dsDNA of bacteria or viruses. Activation of AIM-2 results in the formation of AIM-2 inflammasome and production of pro-inflammatory cytokines and type I interferons.

**From a clinical viewpoint,** pattern recognition receptors (PRRs) are important to induce inflammation, which in certain cases may lead to tissue damage and promote an excess of pro-inflammatory cytokines production up to toxic shock syndrome. Pattern recognition receptors may be important in the pathogenesis of *autoinflammatory disorders*.

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

Lipopolysaccharide (endotoxin, LPS) of Gram-negative bacteria, DAMP are the ligands for:

1. TLR1.
2. TLR2.
3. TLR4.
4. TLR8.

#### ? Question 2

Viral ssRNA is the ligand for:

1. TLR7 and TLR8.
2. TLR1 and TLR2.
3. TLR4 and TLR5.
4. TLR3 and TLR9.

#### ? Question 3

TLRs contain:

1. Carbohydrate recognition domains (CRDs).
2. PYD domain.
3. Caspase activation and recruitment domains (CARD).
4. Toll/interleukin1 receptor (TIR) domain.

#### ? Question 4

Cytosol localized PRRs are:

1. A NLR and RLR.
2. A TLR and CLR.
3. A CLR and ALR.
4. A type I CLR.

#### ? Question 5

TLRs are expressed on:

1. The cytosol.
2. The cell membranes and endosomes.
3. The cell membranes only.
4. The endosomes only.

**? Question 6**

CLRs are expressed on:

1. The cell membranes and endosomes.
2. The cytosol.
3. The cell membranes only.
4. The endosomes.

**? Question 7**

An inflammasome is responsible for:

1. Phagocytosis.
2. NETosis.
3. Pyroptosis.
4. Apoptosis.

**? Question 8**

NLRs contain:

1. NACHT domains.
2. Carbohydrate recognition domains (CRDs).
3. Toll/interleukin1 receptor (TIR) domain.
4. Caspase activation and recruitment domains (CARD).

**? Question 9**

The ligand for RLRs is:

1. Fungi and mycobacteria.
2. Viral dsRNA.
3. Mannose.
4. Diacyl lipopeptides of Gram-positive bacteria.

**? Question 10**

Which PRRs have leucine-rich repeat (LRR) domains?

1. A type I CLR and type II CLR.
2. A TLR and NLR.
3. A RLR.
4. A ALR.

**? Question 11**

Which PRRs have PYD domains?

1. A TLR and CLR.
2. A RLR and CLR.
3. A NLR and ALR.
4. A type I CLR and type II CLR.

**? Question 12**

These PRRs may recognize both “patterns” and antigens:

1. A TLR and CLR.
2. A NLR and ALR.
3. A NLR and RLR.
4. A NLR only.

**? Question 13**

PRRs may trigger:

1. Reactions of the innate immunity only.
2. Complement activation only.
3. Reactions of the innate immunity and adaptive immune responses.
4. The adaptive immune responses only.

**? Question 14**

A TCR and BCR are related to PRRs:

1. Yes.
2. No.
3. In some cases.
4. In certain infections.

**? Question 15**

TLR5 recognizes:

1. Flagellin of bacterial flagella.
2. Viral dsRNA.
3. Viral ssRNA.
4. Unmethylated CpG nucleotides of bacterial and viral DNA.

**? Question 16**

Which members of CLRs are predominant?

1. Type I CLRs.
2. Type I CLRs and type II CLRs.
3. NLRs.
4. Type II CLRs.

### 1.5.3 Cell Adhesion Molecules (CAM)

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**Definitions**

*Cell adhesion molecules (CAMs)* are the receptors in the enlarged sense. They enable the interplay between cells and molecules required for a variety of immune processes.

Cell adhesion molecules (CAM) are an array of cell surface compounds involved in the cell-to-cell interaction. They provide the first mechanism for such interaction. The second mechanism of the communication, nearby or a long way off, is achieved by soluble factors such as cytokines and chemokines.

There are some families of adhesion molecules.

1. Immunoglobulin superfamily
2. Integrins
3. Selectins
4. Mucosal vascular addressins or mucin-type glycoproteins
5. Tumor necrosis factor (TNF) receptor superfamily
6. Cadherin superfamily
7. Extracellular matrix (ECM) proteins or Link family

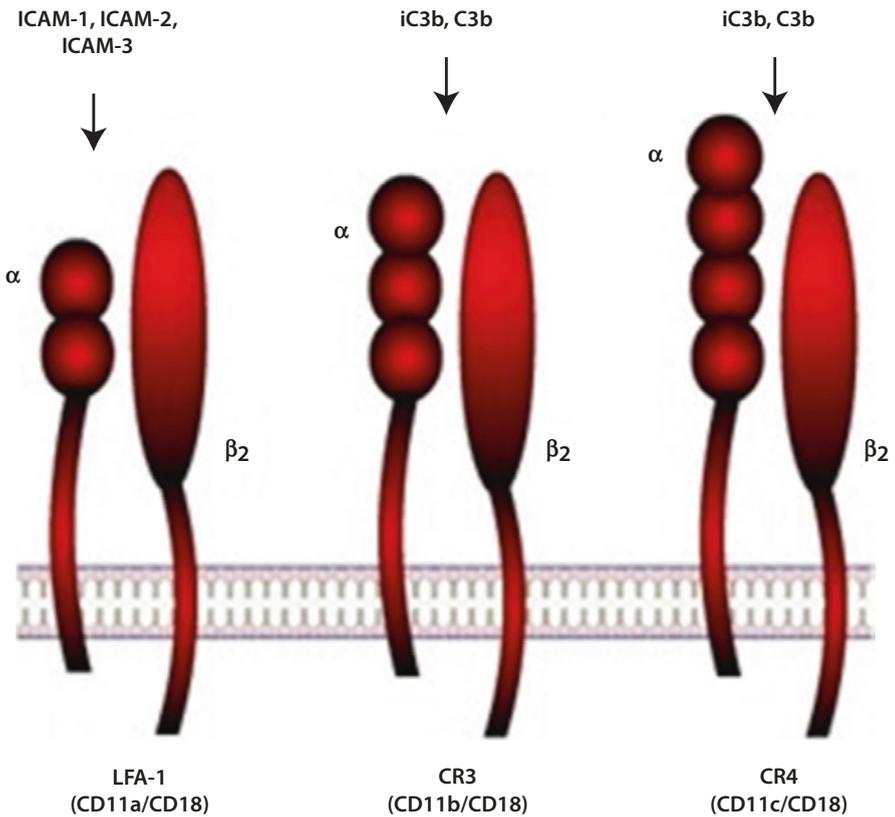
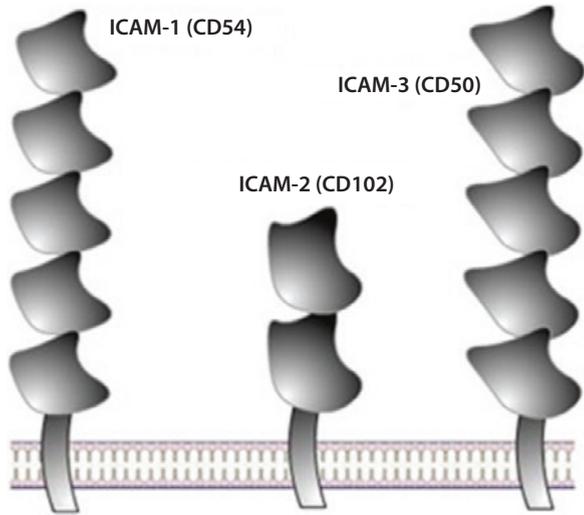
The *immunoglobulin superfamily* comprises approximately 100 members including immunoglobulins, antigen receptors (TCR and BCR), coreceptors (CD4, CD8, CD21), costimulatory molecules (CD28, CTLA-4 (CD152), B7-1 (CD80), B7-2 (CD86)), Fc receptors, HLA molecules, cytokine receptors, PECAM-1 (CD31), ICAM-1 (CD54), ICAM-2 (CD102), and ICAM-3 (CD50). Each immunoglobulin superfamily CAM has one or several extracellular domains, a transmembrane domain, and an intracellular domain that interacts with the cytoskeleton (see ■ Fig. 1.8). The domain motif of sheet organization is stabilized by disulfide bonds. The whole structure enables these CAMs to be resistant to protease attack in the hostile extracellular environment. Ligands for these molecules are antigens, other immunoglobulin superfamily's members, integrins, complement fragments, etc.

PECAM 1 (or CD31) is required for the third stage, direct transmigration, of any complex leukocyte transmigration process through endothelial cells.

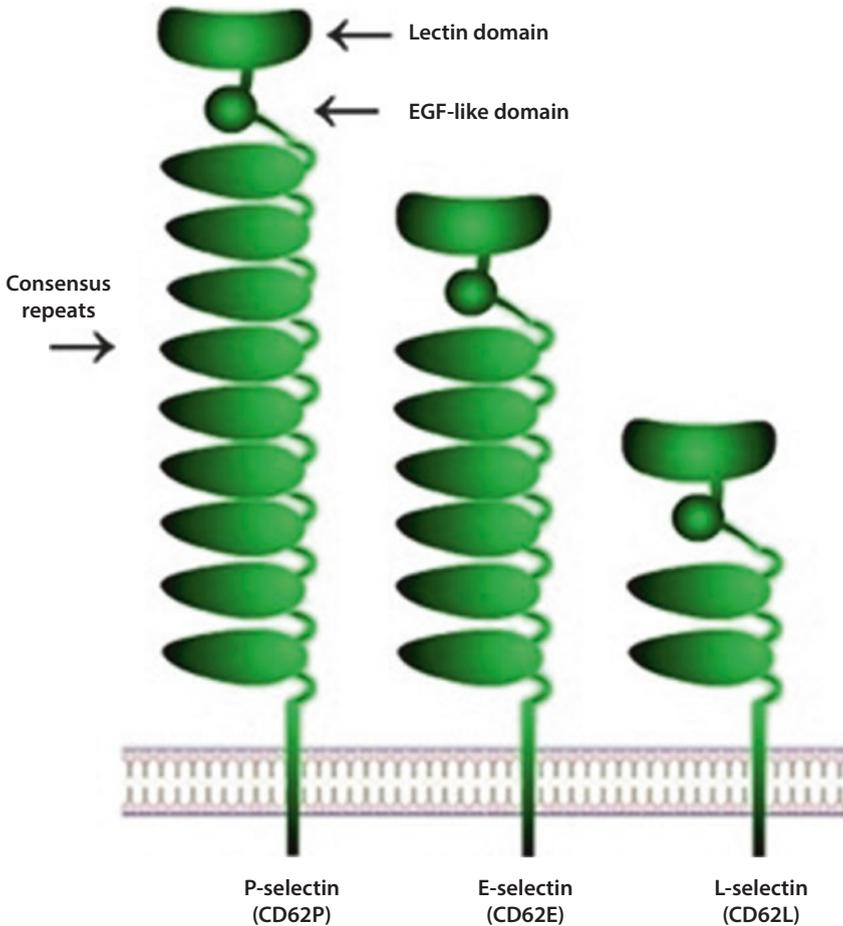
The *integrins* are glycoproteins consisting of two subunits,  $\alpha$  and  $\beta$ . In contrast to  $\alpha$  subunits, the  $\beta$  chains show high homology to each other (see ■ Fig. 1.9). To date, 15  $\alpha$  subunits and 8  $\beta$  subunits have been identified. In most cases, one  $\beta$  subunit combines with several different  $\alpha$  subunits. The integrin family includes LFA-1 ( $\alpha_L\beta_2$ ), complement receptors (CR3,  $\alpha_M\beta_2$ , and CR4,  $\alpha_X\beta_2$ ), VLA-4 ( $\alpha_4\beta_1$ ), etc. An important feature of all integrins is their ability to serve as a bilateral link between the cytoskeleton and the extracellular matrix (ECM). “Outside-in” signaling occurs after the integrin receptor is binding to the ligand, and the signal is transmitted from the integrin receptor into the cell. “Inside-out” signaling occurs when a cell stimulus, for example, triggering of an antigen-recognition receptor/accessory molecule on T or B cell, activates the integrin receptor. This process may activate the integrin to bind its ligand. Ligands for integrins are members of the immunoglobulin superfamily, complement fragments, various serum peptides, etc.

The integrins (LFA 1) along with members of the immunoglobulin superfamily (ICAM 1, ICAM 2, and ICAM 3) play an important role in the second stage, strong adhesion, of the leukocyte transmigration through endothelial cells.

■ Fig. 1.8 Immunoglobulin superfamily molecules



■ Fig. 1.9 Integrin molecules



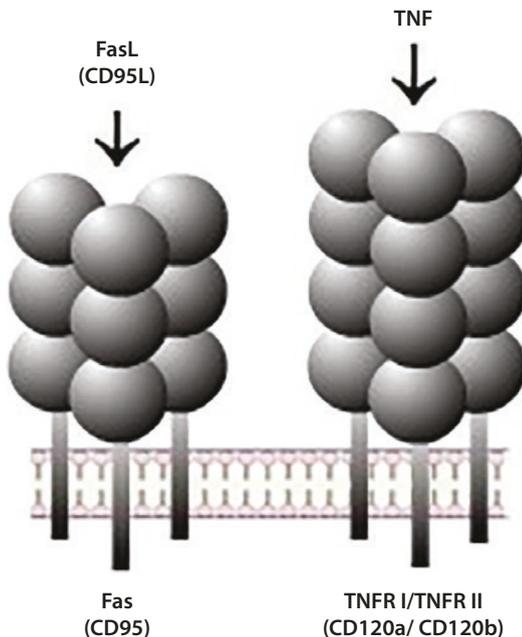
■ Fig. 1.10 Selectin molecules

The *selectin* molecules consist of a ligand-binding lectin domain, epidermal growth factor (EGF)-like domain, some consensus repeats, a transmembrane region, and cytoplasmic tail (see ■ Fig. 1.10). The name “selectin” comes from two words, “selected” and “lectin.”

Selectins (CD62) divide into three subfamilies, platelet-selectin (CD62P), endothelial-selectin (CD62E), and leukocyte-selectin (CD62L), which differ from each other in a number of the consensus repeats and in cell expression, respectively. The selectins play an essential role in the first stage, “rolling” action, of leukocyte transmigration through endothelial cells. They are also involved in inflammatory processes, and cancer progression and metastasis. Ligands for selectins are mucosal vascular addressins (mucin-type glycoproteins).

The *mucosal vascular addressins* or *mucin-type glycoproteins*, ligands for selectins, are extracellular proteins, which carry carbohydrates. Their structure is very

■ Fig. 1.11 Tumor necrosis factor (TNF) superfamily molecules



heterogenic. A major component, which allows them to bind to selectins is sialylated Lewis<sup>x</sup>. The principal function of these CAMs is lymphocyte homing. Sometimes, they are even called the *lymphocyte homing receptors*. Analogous to selectins, vascular addressin PSGL-1 (P-selectin glycoprotein ligand-1) plays an important role in “rolling” action during leukocyte transmigration. The other addressin, GLYCAM-1 (glycosylation-dependent cell adhesion molecule-1), is expressed on the cells of high endothelial venules (HEV) in the lymph nodes and bound to L-selectin. It plays a role in lymphocyte homing and inflammation.

The *tumor necrosis factor (TNF) receptor* superfamily includes CAMs, which can cause apoptosis, inflammation, and costimulatory or coinhibitory effects in the course of adaptive immune responses. The molecules have a certain structural homology. In their active form, the majority of these CAMs complete trimeric complexes on the cell membrane (see ■ Fig. 1.11). They are represented by Fas (CD95), TNFR I/TNFR II (CD120a/CD120b), CD27, CD30, CD40, CD40L, OX40 (CD134), BAFF receptor (CD268), etc. Some of these molecules contain an intracellular “death domain” and are involved in a well-balanced process of apoptosis regulation. The apoptosis plays a crucial role not only in the effector activity of the innate and adaptive immunity but T lymphopoiesis and B lymphopoiesis and immunoglobulin isotype switching.

The *cadherin* molecules are a large polypeptide superfamily, which includes multiple classes of cadherin molecules associated with various tissues (see ■ Fig. 1.12 and ■ Table 1.7). To date, over 100 molecules of cadherins in humans have been identified. Cadherins form adherens junctions to bind cells within the tissues together. The name “cadherin” comes from “calcium-dependent adhesion.” Each molecule has a Ca<sup>++</sup>-dependent extracellular domain, a transmembrane component, and an intracellular cytoplasmic tail associated with

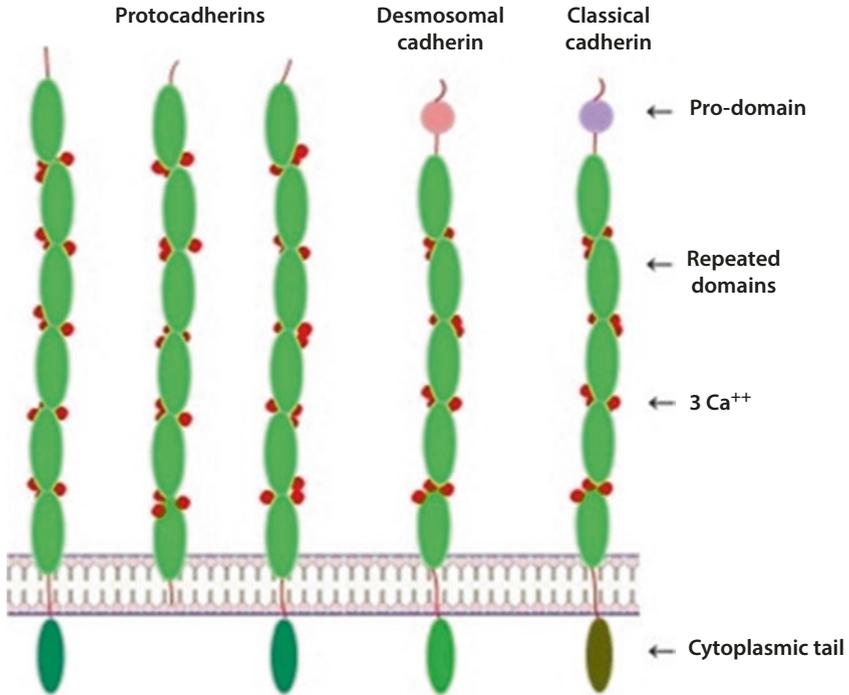
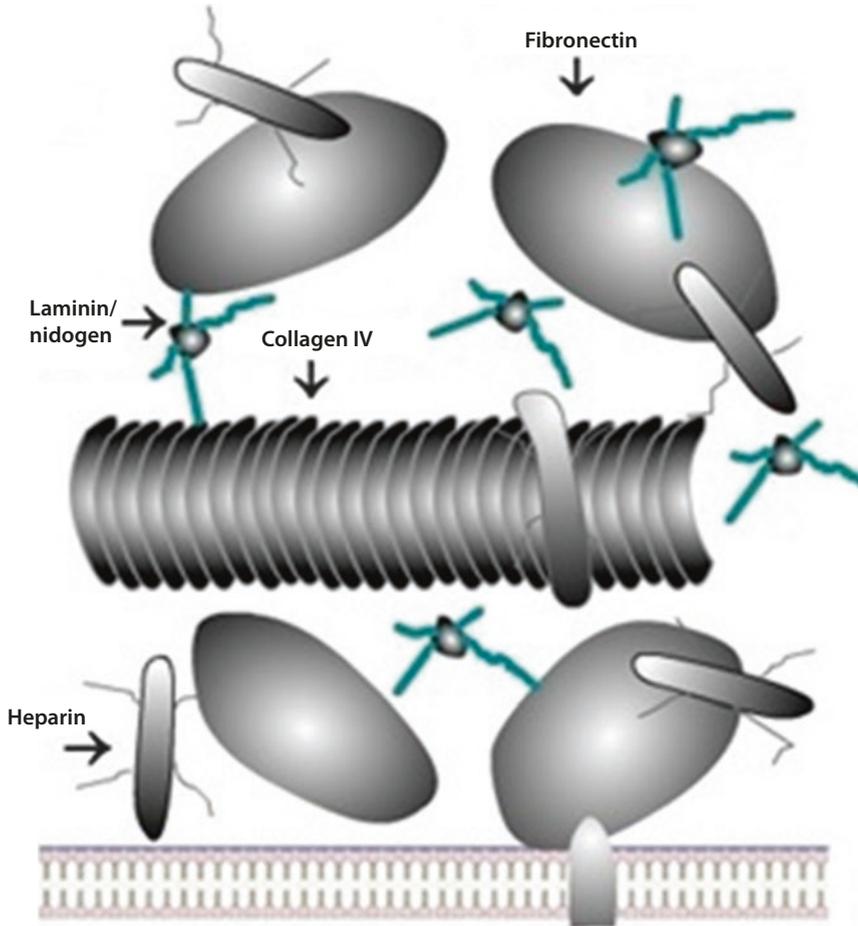


Fig. 1.12 Cadherin molecules

Table 1.7 Samples of various cadherins

Group	Member	Tissue
Classical	CDH1 (E-cadherin)	Epithelium
	CDH2 (N-cadherin)	Neurons
	CDH3 (P-cadherin)	Placenta
Desmosomal	Desmogleins (DSG1, DSG2, DSG3, DSG4)	Skin
	Desmocollins (DSC1, DSC2, DSC3)	Skin
Protocadherins	PCDH1, PCDH10, etc. (a lot)	Various
Ungrouped/unconventional	CDH4 (R-cadherin)	Retina
	CDH6 (K-cadherin)	Kidney
	CDH13 (T-cadherin – H-cadherin)	Heart
	CDH17 (LI-cadherin)	Liver, intestine



■ Fig. 1.13 Molecules of extracellular matrix (ECM)

a large number adapter and signaling proteins. Cadherins have many ligands because they are responsible for the separation of the different tissue layers and for cell movement. For example, loss of E-cadherin's function leads to greater tumor metastasis, and a decrease in desmogleins' activity may result in pemphigus vulgaris.

The *extracellular matrix (ECM)* is the most important complex of molecules by which various cells communicate with each other and fulfill multiple types of their activity. That is why this complex is also called Link family. ECM consists of collagen fibrils, fibronectin, laminin/nidogen complex, and proteoglycans (see ■ Fig. 1.13). ECM's structure is heterogenic and depends on a type of cell that forms the ECM. Ligands for ECM are  $\beta_2$  integrins inside the cells and ECM's components themselves. ECM takes part in stable cell positioning in tissues, the fetal life, cell proliferation, differentiation, migration, regeneration, signaling across the basal membrane, and other types of cell activity. Depending on the

## 1

concentration of ECM's components and their localization on the basal membrane, ECM at the same time may antagonize and upregulate various types of cell behavior.

**From a clinical point of view,** *leukocyte adhesion deficiencies (LAD)* occur in babies and toddlers, and this disorder is characterized by repeated bacterial and fungal infections, intestinal or perianal fistulae, delayed wound healing, and high leukocytosis. Type 1 LAD is associated with CD18 deficiency (mutation on 21q22.3), type 2 LAD is linked to disorder to synthesize sialylated Lewisx (mutation on 11p11.2), and type 3 LAD is caused by mutation in gene (on 11q13.1) that encodes the intracellular protein interacting to  $\beta$  integrins for the provision of “inside-out” signaling in hematopoietic cells.

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

In the enlarged sense, cell adhesion molecules (CAMs) are:

1. Signaling molecules.
2. Cytokines.
3. Chemokines.
4. Receptors.

#### ? Question 2

During the leukocyte transmigration through endothelium, ICAM-1, ICAM-2, and ICAM-3 are important for:

1. Strong adhesion.
2. Direct transmigration.
3. “Rolling” adhesion.
4. Antigen recognizing.

#### ? Question 3

During the leukocyte transmigration through endothelium, integrin LFA-1 is important for:

1. “Rolling” adhesion.
2. Pattern recognition.
3. Strong adhesion.
4. Direct transmigration.

#### ? Question 4

PECAM-1 is related to:

1. Immunoglobulin superfamily CAMs.
2. Integrin CAMs.
3. Selectin CAMs.
4. Mucin-type CAMs.

**? Question 5**

Tumor necrosis factor (TNF) receptor superfamily CAMs are essential for:

1. NETosis.
2. Apoptosis.
3. Pyroptosis.
4. Phagocytosis.

**? Question 6**

Which CAMs contain “death domain”?

1. Integrins.
2. Selectins.
3. TNF receptor CAMs.
4. Cadherins.

**? Question 7**

These CAMs play a role in forming adherens junctions to bind cells together:

1. Selectins.
2. Cadherins.
3. Mucosal vascular addressins.
4. Integrins.

**? Question 8**

The CAMs, which enable to serve as a bilateral link between the cytoskeleton and the extracellular matrix (ECM):

1. Integrins.
2. TNF receptor CAMs.
3. Selectins.
4. Immunoglobulin superfamily CAMs.

**? Question 9**

Very late activation antigen-4 is:

1. ICAM-2.
2. VLA-4.
3. CTLA-4.
4. LFA-1.

**? Question 10**

As a rule, these CAMs consist of three chains:

1. Mucin-type CAMs.
2. TNF receptor CAMs.
3. Adherins.
4. Integrins.

**?** Question 11

These CAMs consist of two chains:

1. Selectins.
2. Cadherins.
3. Integrins.
4. ECM's molecules.

**?** Question 12

Each molecule of these CAMs has a  $\text{Ca}^{++}$ -dependent extracellular domain:

1. Selectins.
2. Cadherins.
3. Integrins.
4. Mucosal vascular addressins.

**?** Question 13

These CAMs comprise proteoglycans and fibronectin:

1. Integrins.
2. Mucosal vascular addressins.
3. Extracellular matrix (ECM).
4. Selectins.

**?** Question 14

Fc receptors are related to:

1. Immunoglobulin superfamily CAMs.
2. Integrin CAMs.
3. Selectin CAMs.
4. Mucin-type CAMs.

**?** Question 15

Desmogleins are related to:

1. Selectins.
2. Integrins.
3. Mucosal vascular addressins.
4. Cadherins.

**?** Question 16

During the leukocyte transmigration through endothelium, selectins and mucin-type glycoproteins are important for:

1. Strong adhesion.
2. Direct transmigration.
3. "Rolling" adhesion.
4. Signaling.

## 1.5.4 Cytokines

### Definitions

*Cytokines* are specialized regulatory molecules, which mainly act on cells in a short distance manner.

*Knockout mice* are often used for studying cytokine functions *in vivo*. A knockout mouse model is a mouse in which a target cytokine gene is deleted or deactivated in the genome of a mouse blastocyst. The loss of gene activity often leads to changes in the phenotype of the adult animal allowing *in vivo* studies of the functions of the target gene.

Most *cytokines* act in a *paracrine* or *autocrine* manner, whereas only a small part of the cytokines may function at the systemic level, by the *endocrine* way, like hormones. They all are low-molecular-weight proteins, peptides, or glycoproteins. The term “cytokine” comes from Greek words “cyto-” and “-kinos,” which means “cell” and “movement.”

Cytokines are produced by almost the entire spectrum of known cell types and characterized by *redundancy* and *pleiotropism*. The concept of “1 producer cell – 1 cytokine – 1 target cell” does not apply to cytokines. There is an enormous number of various cytokines, which act both inside the immune system and throughout the body. Furthermore, cytokine polymorphism is achieved by heredity of allele variants, and during their synthesis, by alternative splicing.

Some cytokine groups may function in both a *synergic* and an *antagonistic* manner and in both a *pro-inflammatory* and an *anti-inflammatory* way. They may affect numerous processes in the immune system including reactions of the innate and adaptive immune responses.

A cytokine acts on a target cell through a high-affinity cytokine receptor triggering subsequent signaling inside the cell. Sometimes, a target cell may not receive cytokine effects because a cytokine may be bound by some serum proteins like albumin or due to the shedding of a cytokine receptor.

There are some classifications of cytokines. Classifications based upon identical or shared biological activities and shared biochemical properties of cytokines are problematic and complex. Families of cytokines share sequence similarity and display homology and some promiscuity in their reciprocal receptor systems.

Historically, the classical nomenclature of cytokines includes:

1. Interleukins (ILs)
2. Colony-stimulating factors (CSFs)
3. Interferons (IFNs)
4. Tumor necrosis factors (TNFs)
5. Transforming growth factors (TGFs)
6. Cytokines and chemokines and a variety of other proteins

**Table 1.8** Cytokine families by sequence similarity

Family	Members
4- $\alpha$ -helix bundle family:	
– IL2 subfamily	IL2, IL4, IL7, IL9, IL13, IL15, IL21
– IL10 subfamily	IL10, IL19, IL20, IL22, IL24, IL26
– Interferon (IFN) subfamily	IFN $\alpha$ , IFN $\beta$ , IFN $\gamma$ , IL28, IL29
IL1 family	IL1 $\beta$ , IL1 $\alpha$ , IL1ra, IL18, IL33, IL36
IL6 family	IL6, IL11, IL31, etc.
IL12 family	IL12, IL23, IL27 (IL30), IL35
IL17 family	IL17A, IL17B, IL17C, IL17D, IL17E (IL25), IL17F
CXCL chemokines family	IL8
TGF $\beta$ superfamily	TGF $\beta$ 1, TGF $\beta$ 2, TGF $\beta$ 3, etc.
CSF family	IL3, IL5, IL34, GM-CSF, G-CSF, M-CSF
TNF superfamily	TNF $\alpha$ , TNF $\beta$ , BAFF
Do not share homology to any cytokine family	IL14, IL16, IL32

Below, you can also see a classification of cytokines, which takes into account sequence similarity and homology (see **Table 1.8**).

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

IL2 belongs to:

1. IL1 family.
2. 4- $\alpha$ -helix bundle family.
3. IL6 family.
4. IL17 family.

#### ? Question 2

IL18 belongs to:

1. IL1 family.
2. TNF superfamily.
3. IL12 family.
4. CSF family.

**? Question 3**

An immunosuppressive cytokine is:

1. IL1.
2.  $\text{IFN}\gamma$ .
3. IL10.
4. GM-CSF.

**? Question 4**

The keystone cytokine of type 1 helper T cells is:

1.  $\text{IFN}\gamma$ .
2. IL4.
3. IL17.
4. M-CSF.

**? Question 5**

The keystone cytokine of type 2 helper T cells is:

1. IL1.
2. IL4.
3. IL35.
4.  $\text{IFN}\gamma$ .

**? Question 6**

Which interleukin is also chemokine?

1. IL2.
2.  $\text{IFN}\alpha$ .
3. IL8.
4. IL9.

**? Question 7**

A pro-inflammatory cytokine is:

1. IL10.
2.  $\text{TGF}\beta$ .
3. IL12.
4. IL35.

**? Question 8**

The cytokine with structural and functional similarity to IL2 is:

1. IL15.
2. IL6.
3. IL8.
4. IL9.

**? Question 9**

This cytokine is identical to IL17E:

1. IL12.
2. IL25.
3. IL4.
4.  $\text{TNF}\alpha$ .

**? Question 10**

Toxic shock syndrome may be caused by the overproduction of:

1. IL10.
2. IL35.
3. IL6.
4. IFN $\alpha$ .

**? Question 11**

Activation of eosinophils may be caused by:

1. IL8.
2. IL1.
3. IL5.
4. TGF $\beta$ .

**? Question 12**

Janus kinase (JAK) family of tyrosine kinases serve for signaling for:

1. Type I and type II cytokine receptors.
2. TNF receptors.
3. BCRs.
4. TGF $\beta$  receptors.

**? Question 13**

A pro-inflammatory cytokine is:

1. TGF $\beta$ .
2. IL35.
3. IL6.
4. IL10.

**? Question 14**

The cytokine with functional similarity to IL4 is:

1. IL7.
2. IL13.
3. TGF $\beta$ .
4. TNF $\beta$ .

**? Question 15**

The keystone cytokine of follicular helper T cells is:

1. IL21.
2. IL17.
3. IFN $\gamma$ .
4. GM-CSF.

**? Question 16**

An immunosuppressive cytokine is:

1. IL8.
2. G-CSF.
3. IL35.
4. IL6.

### 1.5.4.1 Interleukins (ILs)

*IL1 $\beta$* , the soluble IL1, is produced by many cell types, may act at the systemic level like a hormone, exerts the property of endogenous pyrogen, and takes part in the “acute phase” inflammation, costimulation, proliferation, and differentiation of lymphocytes and other cells.

IL1 may be secreted by macrophages in response to “patterns” to result in toxic shock syndrome. IL1 $\beta$  constitutes the IL1 family of cytokines.

*IL $\alpha$* , the membrane IL1, is synthesized by a wide variety of cells, may function in the same manner as IL1 $\beta$ , but also plays an essential role in the maintenance of the skin barrier. It is a member of the IL1 family of cytokines.

*IL1ra (IL1 receptor antagonist)* competes with IL1 for the IL1 receptor and downregulates IL1 activity, i.e., exerts anti-inflammatory effects. It is a member of the IL1 family of cytokines.

*IL2* is secreted by T cells, upregulates T-cell and B-cell growth in the course of the adaptive immune responses, during clonal expansion, and activates NK cells as cells of the innate immunity. IL2 constitutes the IL2 subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL3*, a “multi-colony-stimulating factor,” stimulates the leukopoiesis at early stages. IL3 is a member of the CSF family of cytokines.

*IL4* is produced by type 2 helper T (Th2) cells, mast cells, and B cells. It is the key-stone cytokine of Th2 and IgE antibody synthesis. Analogous to IL2, IL4 plays a role in the advanced B-cell-mediated immune response. IL4 is a member of the IL2 subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL5* refers to cytokines of the Th2 profile and upregulates the eosinophil generation, maturation, and activation. Historically, IL5 was originally discovered as an eosinophil colony-stimulating factor (E-CSF). IL5 is a member of the CSF family of cytokines.

*IL6* is produced by many cell types and also belongs to the Th2 profile. Analogous to IL1, IL6 may act at the systemic level like a hormone and takes part in the “acute phase” inflammation and in the maintenance of the blood-brain barrier. IL6 may also be secreted by macrophages in response to “patterns” to lead to the toxic shock syndrome. IL6 constitutes the IL6 family of cytokines.

*IL7* is a hemopoietic growth factor secreted by stromal and other cells in the bone marrow. IL7 upregulates the differentiation of multipotent stem cells into lymphoid stem cells, which are very important for both B lymphopoiesis and T lymphopoiesis. IL7 is a member of the IL2 subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL8 (CXCL8)* refers to chemokines. IL8 is responsible for the migration of neutrophils toward the site of infection, activation of phagocytosis including respiratory burst, and angiogenesis.

*IL9* is secreted by T cells and belongs to the type 9 helper T (Th9) cells profile. IL9 may activate mast cells, eosinophils, and epitheliocytes and induce inflammation. IL9 is a member of the IL2 subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL10* is a keystone anti-inflammatory and immunosuppressive cytokine, but it belongs to the Th2 profile. IL10 is also produced by natural T regulatory (nTreg) cells, mast cells, and B cells, which downregulates type 1 helper T (Th1) cells, and the expression of HLA molecules and costimulatory molecules required for the adaptive immune responses. IL10 constitutes the IL10 subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL11* is a multifunctional cytokine derived from stromal cells. It takes part in hemopoiesis including thrombocytopoiesis. *IL11* is probably a regulator of placentation and decidualization in pregnant women. It is a member of the *IL6* family of cytokines.

*IL12* is produced by antigen-presenting cells and NK cells to stimulate the Th1 formation during T-cell-mediated responses. *IL12* upregulates the activity of cytotoxic CD8<sup>+</sup>T cells and NK cells due to the production of IFN $\gamma$  and TNF $\alpha$  by these cells. *IL12* refers to pro-inflammatory cytokines. *IL12* constitutes the *IL12* family of cytokines.

*IL13* is related to cytokines of the Th2 profile, which act like *IL4*. *IL13* enhances atopic allergic inflammatory processes even more than *IL4*. However, *IL13* may induce matrix metalloproteinases in the airway to protect lungs from excessive inflammatory proteins, which prevents asphyxiation in asthma. *IL13* is a member of the *IL2* subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL14* is produced by T cells, downregulates the antibody synthesis, and upregulates the generation and maintenance of normal memory B cells. It does not share homology to any cytokine family.

*IL15* is a cytokine with structural and functional similarity to *IL2*, which upregulates the T-cell and NK cell proliferation and activity. *IL15* is a member of the *IL2* subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL16* is produced by a variety of cells and characterized by functional pleiotropism. In particular, it upregulates the CD4<sup>+</sup> T lymphocyte migration. It does not share homology to any cytokine family.

*IL17* is a keystone cytokine of type 17 helper T cells and produced by some CD4<sup>+</sup>T cells under the influence of *IL23*. *IL17* is a pro-inflammatory cytokine involved in the chronic inflammation with the participation of neutrophils and other cells and in autoimmune disorders. *IL17* acts synergistically with TNF $\alpha$ , TNF $\beta$ , *IL1*, and *IL6*. *IL17* constitutes the *IL17* family of cytokines, which includes *IL17A*, *IL17B*, *IL17C*, *IL17D*, *IL17E* (*IL25*), and *IL17F*.

*IL18* is released by macrophages and other cells, promotes the secretion of IFN $\gamma$ , which upregulates macrophages themselves, and stimulates T-cell-mediated responses in a synergistic manner with *IL12*. It is a member of the *IL1* family of cytokines.

*IL19* is secreted by resting monocytes and B cells and upregulates their activity in an autocrine manner. It is a member of the *IL10* subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL20* is produced by activated keratinocytes and monocytes and may play a role in the epidermal function and pathogenesis of psoriasis. It is a member of the *IL10* subfamily of 4 $\alpha$ -helix bundle family of cytokines.

*IL21* is a keystone cytokine of follicular helper T cells (T<sub>fh</sub>). *IL21* is important in the advanced B-cell-mediated response, in particular B-cell clonal expansion, antibody switching, and memory B-cell formation. Also, *IL21* is secreted by Hodgkin's lymphoma tumor cells. On the other hand, *IL21* treatment was approved for a clinical trial in metastatic melanoma, renal cell carcinoma, and *HIV*. *IL21* is a member of the *IL2* subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL22* is produced by dendritic cells, Th17, type 22 helper T (Th22) cells, and splenocytes. *IL22* is a keystone cytokine of Th22, may exert both anti-inflammatory and pro-inflammatory qualities, and plays a role in skin and mucosal immunity as well as in liver functioning to promote hepatocyte survival. It is a member of the *IL10* subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL23*, a pro-inflammatory cytokine, upregulates Th17 formation. It is a member of the IL12 family of cytokines.

*IL24* is secreted by monocytes and macrophages and plays a role in wound healing and antitumor immunity in the skin, lung, and reproductive organs. It is a member of the IL10 subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL25* is IL17E.

*IL26* is produced by Th17, exerts both antibacterial and autoimmune qualities, and can upregulate the IFN $\alpha$  and IFN $\beta$  secretion by plasmacytoid dendritic cells (pDC) with costimulation of TLR. It is a member of the IL10 subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL27* is released by antigen-presenting cells as a regulator of T and B cells. It is a member of the IL12 family of cytokines.

*IL28* is an immunoadjuvant cytokine useful in vaccination against viruses like flu viruses as it modulates the activity of cytotoxic CD8+T cells and production of IFN $\gamma$ . It is a member of the interferon (IFN) subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL29* plays a role in defending against viruses analogous to IL28. It is a member of the IFN subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IL30* is IL27.

*IL31* is produced by CD4+T cells. IL31 may play a role in skin inflammation. IL31 belongs to the IL6 family of cytokines.

*IL32* is a pro-inflammatory cytokine, which induces the production of IL6, IL8 (CXCL8), TNF $\alpha$  and CXCL2, and osteoclast differentiation. It does not share homology to any cytokine family.

*IL33* is produced by a variety of cell types, refers to cytokines of the Th2 profile and ILC group 2, and plays a role in atopic allergic inflammation such as asthma, allergic rhinitis, atopic dermatitis, etc. IL33 is a member of the IL1 family of cytokines.

*IL34* is secreted in the spleen and many organs. IL34 is probably involved in monopoiesis and monocyte activity.

IL34 shares structural homology with the macrophage colony-stimulating factor (M-CSF). It is a member of the CSF family of cytokines.

*IL35* is a keystone anti-inflammatory and immunosuppressive cytokine. IL35 is secreted by nTreg. However, IL35 belongs to the IL12 family of cytokines.

*IL36* regulates CD4+T cells in the skin to promote releasing IL2. IL36 is a member of the IL1 family of cytokines.

*BAFF* (*B-cell activating factor*) is produced by B cells and antigen-presenting cells. BAFF acts as a potent activator of the B-cell differentiation and antibody synthesis and a modulator of B-cell apoptosis. It belongs to the TNF superfamily.

*TGF $\beta$*  (*transforming growth factor- $\beta$* ) is a keystone anti-inflammatory and immunosuppressive cytokine, which is produced by nTreg, type 3 helper T (Th3) cells, and a variety of cell types. Besides, TGF $\beta$  upregulates the angiogenesis, tissue regeneration, embryonic development, and the growth of some human cancer cells.

#### 1.5.4.2 Colony-Stimulating Factors (CSFs)

*Granulocyte-macrophage colony-stimulating factor* (GM-CSF) is secreted by a variety of cell types and stimulates stem cells in the bone marrow to produce granulocytes and monocytes. Analogous to IL1, IL6, TNF $\alpha$ , and TNF $\beta$ , GM-CSF may function at the

systemic level and affect mature cells of the immune system. GM-CSF acts in a synergic manner with IL3.

*Granulocyte colony-stimulating factor (G-CSF)* is secreted by a variety of cell types and stimulates stem cells in the bone marrow to produce granulocytes and release granulocytes and stem cells into the bloodstream. Next, G-CSF upregulates the proliferation, maturation, and survival of granulocytes.

*Macrophage colony-stimulating factor (M-CSF)* is released by some cell types and stimulates the proliferation, differentiation, survival, and functional activity of monocytes and macrophages. M-CSF acts synergistically with IL34. In addition, M-CSF takes part in processes associated with fertility and pregnancy.

### 1.5.4.3 Interferons (IFNs)

*IFN $\alpha$*  is produced by leukocytes and other cell types and takes part in the innate immunity as a potent antiviral agent to promote the cytolysis of target cells. *IFN $\alpha$*  is an anti-inflammatory cytokine and a stimulator of NK cell and macrophage activity. It belongs to type I of the IFN subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IFN $\beta$*  is secreted by fibroblasts and other cell types. It exerts the same effects as *IFN $\alpha$* , in particular, in defense against viral infections. Also, *IFN $\beta$*  is used in the treatment for some forms of multiple sclerosis. It also belongs to type I of the IFN subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

*IFN $\gamma$*  is produced by lymphocytes upon their activation, belongs to the Th1 profile of cytokines, and exhibits wide immunoregulatory qualities in the immune processes. As contrasted to type I *IFN $\alpha$*  and *IFN $\beta$* , *IFN $\gamma$*  is a pro-inflammatory cytokine, which may act in a synergic manner with *TNF $\alpha$* , *TNF $\beta$* , IL1, IL6, and other cytokines and chemokines. *IFN $\gamma$*  is related to type II of the IFN subfamily of the 4 $\alpha$ -helix bundle family of cytokines.

The interferon effects will be described in detail in ► Chap. 3.

### 1.5.4.4 Tumor Necrosis Factors (TNFs)

*TNF $\alpha$*  is produced by macrophages (M1) and a variety of cell types, “cachectin,” which has many functions. *TNF $\alpha$*  acts synergistically with *TNF $\beta$* , IL1, IL6, and *IFN $\gamma$* . It is a potent pro-inflammatory cytokine and even endogenous toxin, an endogenous pyrogen, and a regulator of adaptive immune responses. Historically, *TNF $\alpha$*  was discovered and named in such manner as could lyse particular tumor cell lines.

*TNF $\beta$*  (“lymphotoxin”) is secreted by lymphocytes. Its effects are similar to *TNF $\alpha$* ’s effects, but *TNF $\beta$*  is more critical for the development of lymphoid tissue.

### 1.5.4.5 Cytokine Receptors

Cytokines act on their target cells by binding specific cytokine membrane receptors. The receptors are divided into several families based on their structure and activities and further categorized into many subgroups. Cytokine receptors are the cell adhesion molecules (CAM), which belong to different CAM’s families such as the immunoglobulin superfamily, TNF receptor superfamily, TGF $\beta$  receptor superfamily, etc.

Type I cytokine receptors have certain conserved motifs in their extracellular domain and serve as receptors for some interleukins and CSFs.

Type II cytokine receptors are multimeric receptors composed of heterologous subunits and serve as receptors for some interleukins and interferons.

Type I and type II cytokine receptors, for signaling, are connected to the Janus kinase (JAK) family of tyrosine kinases.

TNF receptor superfamily members share a cysteine-rich domain formed of three disulfide bonds. Some of them contain a cytoplasmic death domain associated with procaspases through adapter proteins, which can cleave other inactive procaspases and trigger the caspase cascade and cell apoptosis.

TGF $\beta$  receptors are polymorphic and can be homo- or heterodimeric. They use, for signaling, serine/threonine kinase-dependent pathways.

**From a clinical point of view,** there are many clinical conditions associated with cytokine effects, which may be both positive and negative. Oversecretion of cytokines can trigger dangerous autoinflammatory syndromes known as a *cytokine storm* as well as *toxic shock syndrome*. On the other hand, specific activities of some cytokines have been the basis for therapeutical intervention, in particular for the treatment of malfunctions of hemoipoiesis and tumor therapy. Current concepts use the support of chemo- and radiotherapy, bone marrow transplantation, and approaches to immune enhancement therapy.

## 1.5.5 Chemokines

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### Definitions

*Chemokines* are specialized cytokines, which drive the directed migration of immune system's cells for homing and/or inflammation. Chemokines are divided into *homeostatic* and *inflammatory* chemokines.

As opposed to classic leukocyte chemoattractants, which have little specificity, *chemokines* induce the recruitment of well-defined cell types and leukocyte subsets. For example, CXC chemokines can attract neutrophils but not macrophages, while CC chemokines preferentially induce the migration of macrophages. To date, about 40 chemokines have been identified in humans. They mainly act on neutrophils, monocytes/macrophages, lymphocytes, and eosinophils and play an essential role in host defense mechanisms. In addition, chemokines regulate the lymphoid organ development, the functioning of the nervous system, and may stimulate tumor cell metastasis.

Chemokines have been structurally classified into four main subfamilies, (1) C, (2) CC, (3) CXC, and (4) CX3C, depending on the location of *cysteine* (C) between other amino acids (X) in the chemokine's protein molecule sequence. Addition of the letter L refers to chemokines themselves and means *Ligand*, whereas the letter R is related to chemokine receptors and denotes *Receptor*.

Chemokines may be also functionally divided into two groups, homeostatic and inflammatory.

*Homeostatic chemokines* are produced in certain tissues and cells and are responsible for homing leukocytes in particular organs or tissues. Tissue-specific chemokine receptors are closely associated to various cell adhesion molecules to provide settling organs of the immune system with lymphocytes and other cell types. As compared to inflammatory chemokines, the homeostatic chemokines exert a more diverse range of functions. They may be involved in the organogenesis, migration of progenitor cells, and cell development. However, homeostatic chemokines can also be involved in the carcinogenesis and metastasis of cancer cells.

Homeostatic chemokines include CCL14 (hemofiltrate CC chemokine 1, HCC-1), CCL19 (EBI1 ligand chemokine, ELC; Exodus-3), CCL20 (macrophage inflammatory protein-3 $\alpha$ , MIP-3 $\alpha$ ), CCL21 (secondary lymphoid tissue chemokine, SLC; Exodus-2), CXCL13 (B lymphocyte chemoattractant; BLC), CCL25 (thymus-expressed chemokine, TECK), CCL27 (cutaneous T-cell-attracting chemokine, STACK), CCL28 (mucosae-associated epithelial chemokine, MEC), CXCL12 (stromal cell-derived factor-1, SCDF-1), etc.

*CXCL12 (stromal cell-derived factor-1, SCDF-1)* is produced by reticular cells in large amounts to bind to *CXCR4* on hematopoietic stem cells. *CXCL12* upregulates the homeostatic retention and development of many cell types in the *bone marrow*. In addition, *CXCL12* along with *CCL21* and *CCL25* plays a role in the homing of thymocytes in the *thymus*.

*CCL28 (mucosae-associated epithelial chemokine, MEC)* is produced by columnar epithelial cells in the lung, breast, gut, and salivary glands and provides the *mucosal homing* with T cells and B cells expressing *CCR10* and eosinophils expressing *CCR3*. *CCL28* also participates in the migration of T cells and IgA-producing plasma cells to the exocrine glands, GI tract, and other mucosal sites. It is believed that *CCL28* may exhibit a potential antimicrobial activity against certain pathogens, such as bacteria and fungi. Sequence analysis has revealed *CCL28* to be most similar to *CCL27*.

*CCL27 (cutaneous T-cell-attracting chemokine, CTACK)* is secreted by epidermal basal keratinocytes and epitheliocytes in many tissues. TNF $\alpha$  and IL1 $\beta$  upregulate the *CCL27* production. *CCL27* drives the homing of memory T cells to the *skin* and also plays a role in T-cell-mediated inflammation of the skin. It exerts chemotactic effects by binding to the chemokine receptor *CCR10*, which is expressed by skin CLA+ T cells and vascular endothelial cells, and fibroblasts.

*CCL25 (thymus-expressed chemokine, TECK)* exhibits a unique and highly restricted expression pattern compared with other chemokines. *CCL25* is expressed at high levels primarily in the thymus and small intestine. It is chemotactic for thymocytes, macrophages, and dendritic cells during the homing of these cells to the *thymus* and *small intestine*. *CCL25* exerts its effects by binding to the chemokine receptor *CCR9*. In addition, *CCL25* plays a role in the development of the small intestinal  $\gamma\delta$ T cells.

*CCL21 (secondary lymphoid tissue chemokine, SLC; Exodus-2)* is expressed in the *lymph nodes*, *spleen*, and *appendix* and chemotactic for thymocytes, activated T cells, and dendritic cells. Correspondingly, *CCL21* also upregulates homing of the thymocytes in the *thymus*. This chemokine elicits its effects by binding to the chemokine receptor *CCR7*.

*CCL19 (EBI1 ligand chemokine, ELC; Exodus-3)* is expressed abundantly in the thymus and lymph nodes providing trafficking of central memory T (T<sub>CM</sub>) cells, B cells,

and dendritic cells to the *secondary lymphoid organs*. This chemokine elicits its effects by binding to the chemokine receptor *CCR7*.

*CXCL13* (*B lymphocyte chemoattractant, BLC*) promotes the homing of B cells in follicles of the *secondary lymphoid tissues* and appears to form Tfh. *CXCL13* interacts with *CXCR5*.

*Inflammatory chemokines* are constituted under pathological conditions due to pro-inflammatory stimuli from sites of infection, injury, or tissue damage and take an active part in the inflammatory response attracting a wide variety of cells in both the innate and adaptive immunity. Under the influence of inflammatory chemokines, cells will extravasate from the blood vessel and follow the gradient to pro-inflammatory stimuli. This group of chemokines is also recruited in wound healing.

Inflammatory chemokines include *CXCL8* (*IL8*), *CCL2* (macrophage chemoattractant protein-1, *MCP-1*), *CCL8* (macrophage chemoattractant protein-2, *MCP-2*), *CCL7* (macrophage chemoattractant protein-3, *MCP-3*), *CCL3* (macrophage inflammatory protein-1 $\alpha$ , *MIP-1 $\alpha$* ), *CCL4* (macrophage inflammatory protein-1 $\beta$ , *MIP-1 $\beta$* ), *CCL5* (regulated on activation, normal T-cell expressed and secreted, *RANTES*), *CCL11* (*Eotaxin-1*), *CCL24* (*Eotaxin-2*), *CXCL10* (*IFN $\gamma$ -induced protein-10, IP-10*), etc.

Some chemokines may be simultaneously related to both groups.

#### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

Which interleukin is also chemokine?

1. IL2.
2. IFN $\alpha$ .
3. IL8.
4. IL9.

#### ? Question 2

The chemokine, which is essential for homing of many cells in the central organs of the immune system, is:

1. CCL28.
2. CXCL12.
3. CCL27.
4. CXCL13.

#### ? Question 3

The chemokine, which is important for homing of T cell in the skin, is:

1. CXCL12.
2. CCL21.
3. CCL28.
4. CCL27.

**? Question 4**

Which chemokine is inflammatory?

1. CCL2.
2. CXCL12.
3. CCL28.
4. IL1.

**? Question 5**

CXC-chemokine molecules consist of:

1. Four cysteine molecules going in turn.
2. Two cysteine molecules, which contain another amino acid molecule in-between.
3. Two cysteine molecules, which contain three amino acid molecules in-between.
4. Two cysteine molecules going in turn.

**? Question 6**

Chemokine always comprise:

1. Nucleotides.
2. RNA.
3. Cysteine molecule/molecules.
4. Saccharides.

**? Question 7**

Chemokines are:

1. Nucleic acids.
2. Polysaccharides.
3. Proteins.
4. Fatty acids.

**? Question 8**

A typical chemokine receptor is linked with:

1. G-protein.
2. JAK tyrosine kinases.
3. MyD88 adapter protein.
4. Y domain.

**? Question 9**

A chemokine receptor is:

1. IL2R.
2. CCR10.
3. TCR.
4. TLR.

**? Question 10**

Homeostatic chemokines are responsible for:

1. Inflammatory process only.
2. Inflammatory process in any cases.

3. Homing of leukocytes.
4. NETosis.

**?** Question 11

Chemokines may promote tumor cell metastasis:

1. No.
2. Never.
3. In certain cases.
4. Always.

**?** Question 12

Macrophage chemoattractant protein-1 is:

1. CCL2.
2. CCL3.
3. CCL5.
4. CCL24.

**?** Question 13

RANTES is:

1. CCL4.
2. CCL19.
3. CCL5.
4. CXCL13.

**?** Question 14

CX3C-chemokine molecules consist of:

1. Four cysteine molecules going in turn.
2. Two cysteine molecules, which contain another three amino acid molecules in-between.
3. Three cysteine molecules going in turn.
4. Two cysteine molecules going in turn.

**?** Question 15

Inflammatory chemokines take an active part in:

1. Apoptosis.
2. Cell homing.
3. Inflammation.
4. NETosis.

**?** Question 16

The chemokine, which is important for mucosal homing of T cells and B cells, is:

1. CXCL12.
2. CCL27.
3. CXCL8.
4. CCL28.

### 1.5.5.1 Chemokine Receptors

The action of any chemokine is mediated when it interacts with a chemokine receptor that has seven transmembrane domains. A typical chemokine receptor is linked to G-protein through which it performs signaling. The chemokine receptors are categorized into the same four subfamilies as chemokines themselves.

**From a clinical viewpoint,** chemokine receptors CCR5 and CXCR4 are coreceptors for CD4 molecule during the *HIV*'s attachment process to the CD4+T cells.

## 1.6 Organs of the Immune System

### Definitions

The *thymus* is a *primary immune organ* in which thymocytes are differentiated into T cells, and a variety of the immunoregulatory substances are produced.

The *bone marrow* is a *primary immune organ* that is the source of all cell lines used by the immune system and the place of B-cell maturation.

The *spleen* is a *secondary lymphoid organ* where defensive immune processes proceed if pathogens invade the body through the blood.

*Lymph nodes* are *secondary lymphoid organs* in which adaptive immune responses and other processes take place if pathogens invade the body through barrier tissues.

*Tertiary lymphoid organs* are newly constituted pathological lymphoid aggregates at the sites of chronic inflammation.

Normally, the immune system is composed of primary (or central) and secondary (or peripheral) organs. Analogous to the whole body, these organs are not quite anatomically symmetrical. The *primary organs*, which include the thymus and bone marrow, enable the performance of functions of the cell commitment and immunoregulation. The *bone marrow* is the “maternity home” for all types of cells and is the “academy” for *B cells*. The *thymus* represents the “immunological government” to master the whole immune system and plays a role as the “academy” for *T cells*. CXCL12 (SCDF-1) is a potent chemokine responsible for the homing of lymphocytes in the primary lymphoid organs.

The protective reactions and responses proceed in the *secondary organs* such as the lymph nodes, spleen, lymphatics, and mucosae-associated lymphoid tissue (MALT) divided into nasal-associated lymphoid tissue (NALT), tube-associated lymphoid tissue (TALT), bronchus-associated lymphoid tissue (BALT), gut-associated lymphoid tissue (GALT), and skin-associated lymphoid tissue (SALT).

A variety of competent immunological islets are present in other organs (e.g., the liver, kidneys, synovia, etc.). *Tertiary lymphoid tissues or organs* are pathologically ectopic accumulations of lymphoid cells and various cell types at the sites of the unresolved immune responses and chronic inflammation such as autoimmune and autoinflammatory disorders, chronic infectious diseases, lymphedema, some types of cancer, and graft

rejection in transplantation. Tertiary lymphoid aggregates may arise at undetermined locations of peripheral tissues: the skin, salivary glands, joints, GI tract, etc. Newly constituted lymphatic and blood vessels and high endothelial venules (HEV) are major structural features of the tertiary lymphoid tissues, whereas the cellularity and involvement of cytokines and chemokines are not yet fully understood. They are a fertile area of research in immunopathology.

**From a clinical viewpoint,** it is supposed that the tertiary lymphoid organs are of crucial importance for the prevention and treatment for a wide range of diseases.

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

The immune organ in which T lymphopoiesis proceeds:

1. Bone marrow.
2. Thymus.
3. MALT.
4. Spleen.

#### ? Question 2

The immune organ in which B lymphopoiesis proceeds:

1. Thymus.
2. Appendix.
3. Isolated follicles.
4. Bone marrow.

#### ? Question 3

A secondary lymphoid organ that functions at the systemic level is:

1. Appendix.
2. Bone marrow.
3. Thymus.
4. Spleen.

#### ? Question 4

A primary immune organ is:

1. Bone marrow.
2. Spleen.
3. Appendix.
4. MALT.

#### ? Question 5

A compartment of MALT is:

1. Thymus.
2. NALT.
3. Skin.
4. A lymph node.

**? Question 6**

This cell takes part in forming microenvironment for thymocytes in the thymus:

1. B cell.
2. Mast cell.
3. Giant epithelial “nurse” cell.
4. Splenocyte.

**? Question 7**

The fluid, which is constituted during the absorption into the lymphatic system, is:

1. Blood.
2. Saliva.
3. Lymph.
4. Gingival clevicular fluid.

**? Question 8**

Lymph nodes paracortical zone is the place of:

1. T cells.
2. B cells.
3. Follicular helper (Tfh) T cells.
4. Plasma cells.

**? Question 9**

Lymph nodes follicles are the zone of:

1. Plasma cells.
2. B cells.
3. All subsets of T cells.
4. Mast cells.

**? Question 10**

Spleen’s white pulp consists of:

1. Erythrocytes and macrophages.
2. Lymphoid follicles and PALS.
3. Platelets.
4. Basophils and mast cells.

**? Question 11**

Reactions of the innate immunity and adaptive responses take place in:

1. “Red” bone marrow.
2. Thymus.
3. Secondary lymphoid organs.
4. “Yellow” bone marrow.

**? Question 12**

Tertiary lymphoid organs are described in:

1. Unresolved inflammatory processes.
2. Healthy adults.
3. Healthy babies and toddlers.
4. Healthy teenagers.

**? Question 13**

A potent chemokine important for the homing of cells in the primary immune organs is:

1. CCL28.
2. CCL27.
3. CXCL12.
4. CCL19.

**? Question 14**

A chemokine does not take part in homing of cells in the thymus is:

1. CXCL12.
2. CXCL13.
3. CCL25.
4. CCL21.

**? Question 15**

The splenectomy may lead to:

1. Increased death rate from pneumonia.
2. Chronic fatigue syndrome.
3. Increased tumor morbidity.
4. Increased formation of memory B cells.

**? Question 16**

Marginal B cells are derived from:

1. Bone marrow.
2. MALT.
3. Thymus.
4. Spleen.

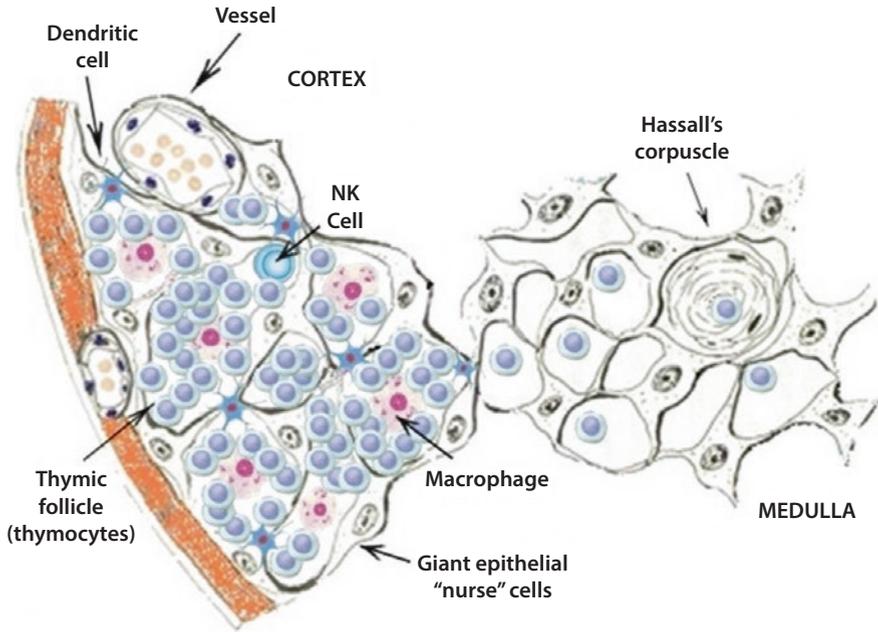
### 1.6.1 The Thymus

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The *thymus* is located in the anterior mediastinum and consists of capsule-coated lobules containing the strictly isolated parenchyma, and the future T cells, thymocytes, which may pass through the vessel endothelium here. There are three parenchymal zones of the thymus, *subcapsular*, *cortical*, and *medullary* (see ■ Fig. 1.14), that accord with major stages of T-cell lymphopoiesis. In the first and second zones, many thymic follicles are available, whereas in the third zone, thymic corpuscles (Hassall's) occur. The role of Hassall's corpuscles is not entirely determined. In the thymus, suspension thymocytes account for approximately 97%, namely, 85% in the cortex and 12% in the medulla. Giant epithelial "nurse" cells, macrophages, thymic dendritic cells, and NK cells make up less than 1% in total. These cells are constant representatives of the thymic microenvironment, "professors," which teach "students," the thymocytes.

T lymphopoiesis will be described in ► Sect. 1.7.2.

The thymus produces numerous hormones, neurotransmitters, and molecules of the immune system such as thymosins, antidiuretic hormone (ADH) or vasopressin, oxytocin, glucocorticoids,  $\beta$  endorphins, enkephalins, cytokines (IL1, IL2, IL3, IL6,



■ Fig. 1.14 Structure of the thymus

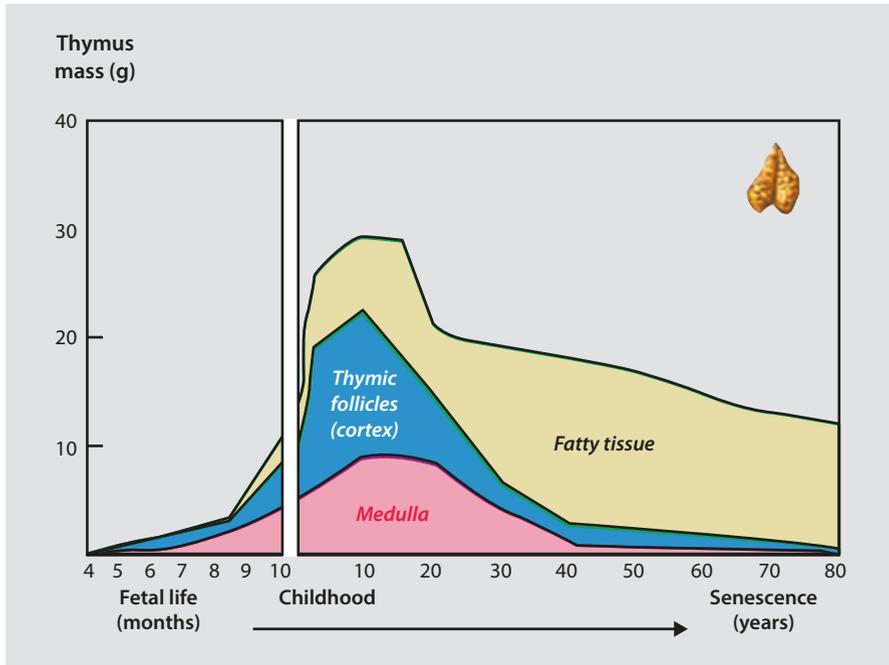
IL7, IL9, GM-CSF, etc.), and chemokines that make up the microenvironment for the thymocytes.

The thymus progressively tends to atrophy starting almost from the birth. It is the so-called *age-dependent thymus involution* (see ■ Fig. 1.15), which corresponds to a prolonged decrease in its endocrine function and a substitution for lipid tissue. This process differs individually. It seems to be the programmed duration of the thymus's ability to perform its strategic homeostatic function that may affect the lifespan.

**From a clinical point of view,** the prolonged abuse of drugs including exogenous opioids because of the endogenous  $\beta$  endorphin system's exhaustion and oxidative stress due to the accumulation of reactive oxygen species (ROS) may result in the accelerated thymic involution, progressive thymic atrophy, and early aging of the whole body. A thymectomy performed in adults does not lead to any immunodeficiency. On the other hand, in clinical practice, many thymic products are used for immune enhancement therapy.

## 1.6.2 The Bone Marrow

There are two types of *bone marrow*, “red marrow,” which is made up of hematopoietic tissue including cells of the immune system, and “yellow marrow,” which consists of fat cells. The bone marrow's pluripotent stem cells can be differentiated into a variety of cell types, e.g., lymphoid stem cell.



■ Fig. 1.15 Age involution of the thymus

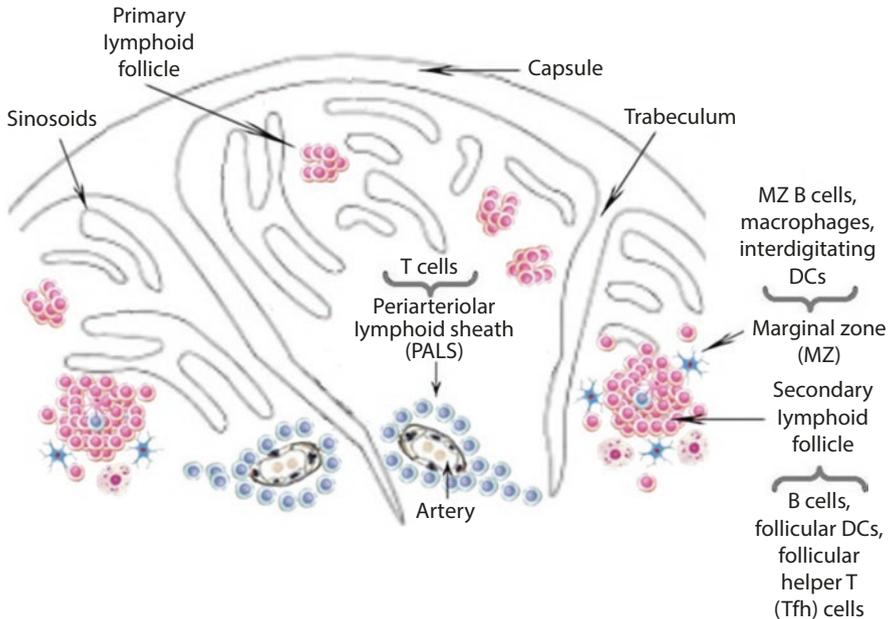
The bone marrow's stroma provides the hematopoietic microenvironment that facilitates hematopoiesis, in particular, B-cell lymphopoiesis, through the parenchymal cells that produce colony-stimulating factors (CSFs) and other cytokines. Nowadays, it is known that the bone marrow may perform a valve-like function to prevent the back-flow of lymphatic fluid in the lymphatic system. Furthermore, the blood vessels of the bone marrow create a blood-marrow barrier, which inhibits immature cells from leaving the bone marrow.

B lymphopoiesis will be described in ► Sect. 1.7.3.

**From a clinical viewpoint,** the bone marrow is the most important object for transplantation in children with primary immunodeficiencies. Biopsies of the bone marrow widely use for diagnostic purposes in hematology.

### 1.6.3 The Spleen

The *spleen* is one of the secondary organs of the immune system but functions at the systemic level when antigens enter the blood. Thus, the spleen is of importance in fighting infections that have invaded the blood. It is similar in structure to a large lymph node located in the left upper quadrant of the abdomen. However, the spleen



■ Fig. 1.16 Spleen's white pulp

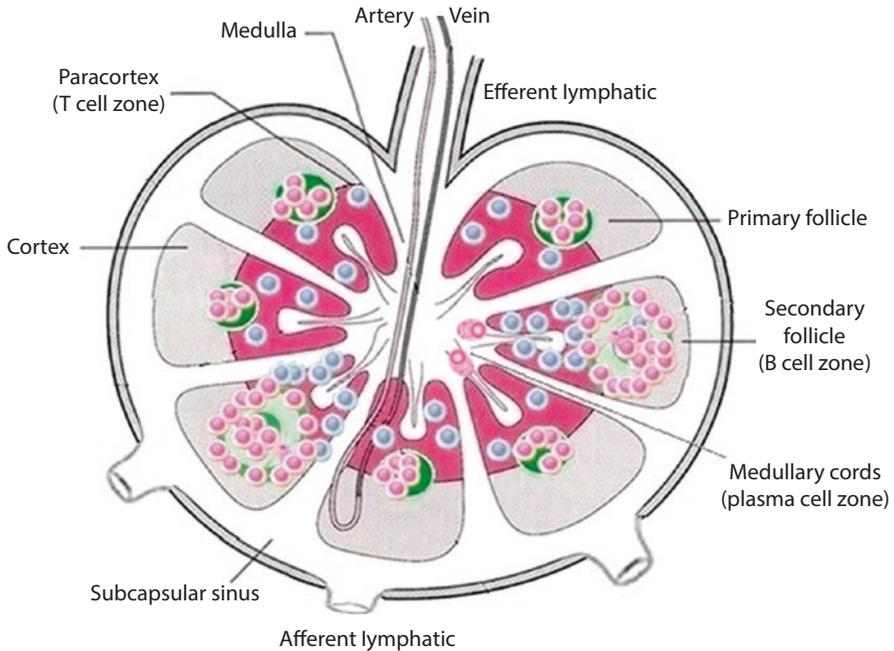
has the splenic artery, splenic vein, and only efferent lymphatic vessels. It consists of red pulp and white pulp. The red pulp plays a role in blood clearance, removing old erythrocytes, maintaining an additional reservoir of blood, and metabolizing hemoglobin.

The white pulp is composed of *lymphoid follicles*, rich in B cells; *marginal zones (MZ)*, rich in MZ B cells; and *periarteriolar lymphoid sheaths (PALS)*, rich in T cells (see ■ Fig. 1.16). The white pulp is vital for the immune processes including B-cell-mediated responses, synthesis of antibodies, removal of antibody-coated microbes, production of properdin, and storage of monocytes.

**From a clinical viewpoint,** a splenectomy leads to a significant increase in the usual death rate from pneumonia, an increase the death rate from ischemic heart disease, and a much diminished frequency of memory B cells. If splenectomy is planning, vaccination against *Pneumococci* must perform.

### 1.6.4 The Lymph Nodes

Some blood fluid from the bloodstream leaks out into tissues and because of the pressure gradient is absorbed into the lymphatic system becoming the *lymph*. In the course of lymph flow, the lymph picks up antigens, antigen-presenting cells, and



■ Fig. 1.17 Structure of the lymph node

lymphocytes throughout the body and carries them via lymphatics into the lymph nodes.

The *lymph nodes* are among the secondary organs of the immune system, widely present in many parts of the body, and have an artery, vein, and afferent and efferent lymphatic vessels. The afferent lymphatics are multiple and wider than efferent vessels, so cells and molecules can easily enter the lymph nodes. Conversely, the passage of large cells like macrophages through the efferent vessels is difficult, so that they remain to function within the lymph node.

Each lymph node is divided into lymph nodules, which contain a cortical zone of *primary follicles* with B cells, a *paracortical zone* of T cells, and a basal part of the nodule in the medulla. The primary follicles develop into *secondary follicles* in the course of B-cell-mediated immune responses. Lymphocytes enter the lymph nodes through specialized high endothelial venules (HEV) found in the paracortical zone (see ■ Fig. 1.17).

**From a clinical point of view** the lymph nodes have essential clinical significance. They may be enlarged, swollen, and inflamed in many infections, tumors, and even under some noninfectious conditions. The palpation of accessible lymph nodes serves as a suitable means of monitoring for any physician.

**Table 1.9** Chemokines and cell adhesion molecules involved in the homing of lymphocytes and other cells in the secondary lymphoid organs

Target sites	Chemokine	Chemokine receptor	Cell adhesion molecules
Compartments of the secondary lymphoid organs	CCL19 (ELC)	CCR7	L-selectin ICAM-1 ICAM-2 VCAM-1 $\alpha_4\beta_1$
	CCL21 (SLC)		
	CXCL13 (BLC)	CXCR5	

There are many chemokines and cell adhesion molecules, which are very important for completing the secondary lymphoid organs by lymphocytes and other cells (see **Table 1.9**).

## 1.7 Cells of the Immune System

### Definitions

*Lymphocytes* and *monocytes*, or agranulocytes, and *polymorphonuclear leukocytes* (PMN) or granulocytes complete **white blood cells (WBCs)** in the human bloodstream. These cells are present in a common blood test.

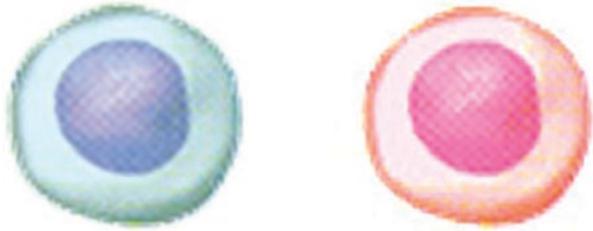
T lymphocytes (T cells) and B lymphocytes (B cells) are the main cells of adaptive responses, whereas innate lymphoid cells (ILCs) are important for innate immunity.

### 1.7.1 Lymphocytes

Lymphocytes are the main cell type among cells of the immune system. By expert estimation, lymphocytes make up about  $10^{13}$  cells in the body. There are T cells, B cells (see **Fig. 1.18**), and innate lymphoid cells (ILCs) including NK cells (see **Fig. 1.19**). Both T cells and B cells undergo differentiation from uncommitted, nonfunctional precursor cells to highly sophisticated effector and memory cells. T lymphopoiesis and B lymphopoiesis proceed in separation. There are two major stages related to lymphocyte maturation, commitment, and priming.

The *commitment* is the differentiation of a lymphoid stem cell into a *naive lymphocyte*, which belongs to a particular clone, has a unique TCR or BCR, and may be referred to as a cell of a certain lymphocyte subset. Naive cells are not antigen-experienced yet. A lymphocyte *clone* is a group of lymphocytes with identical TCR or BCR derived from the same T-cell or B-cell progenitor.

■ Fig. 1.18 T-cell (left) and B-cell



■ Fig. 1.19 Innate lymphoid cell (ILC)



The *priming* is the differentiation of a naive cell during antigen-induced adaptive immune response that results in the formation of an *effector lymphocyte* and *memory lymphocyte*.

Overall, the major qualities of the lymphocytes are as follows.

1. Circulation and recirculation throughout the body via the bloodstream, lymph flow, secretions, and intercellular space
2. The ability to recognize “self,” “non-self,” and “former self” on the base of ligand-receptor interaction, i.e., the ability to learn
3. Clonal diversity (F. Macfarlane Burnet) and network formation (N.K. Jerne)
4. Permanent gene rearrangement in the cells to achieve a precise specificity at any age if an antigen invasion occurs
5. The ability to preserve previously invaded antigens in the memory and enable fast secondary immune responses to prevent the same infection

The subtle, highly specialized hierarchy... The ability to learn and be taught... The capacity to recognize and be tuned in... Be faithful in the protection, but sometimes betray. Have the lymphocytes and immune system some features of intellect?

**From a clinical viewpoint,** lymphocytes, as well as stem cells, are a cellular material for the *cell therapy* in a variety of severe and chronic diseases including cancer. The cell therapy is the basis of current therapeutic benefit in pathology.

In healthy children *since 4–5 days till 4–5 years*, lymphocytes are predominant cells over neutrophils in the periphery. *Lymphocytosis*, the high count of circulating lymphocytes, may be present in acute viral infections, some bacterial infections (e.g., pertussis, tuberculosis, brucellosis, etc.), leukemias, and some other pathological conditions. *Lymphocytopenia*, the diminished level of circulating lymphocytes, may be present in some primary immunodeficiencies, *HIV* infection and some autoimmune disorders and tumors, and as a result of the chemotherapy and radiotherapy for them.

## 1.7.2 T Cells and T Lymphopoiesis

### Definitions

*T cells* play a pivotal role in the T-cell-mediated immune responses and immunoregulation since they have been differentiated in the thymus and in part in the periphery.

There are *CD4+T cells* including a variety of *adaptive helper CD4+ T-cell subsets*, *CD8+T cells*, *natural killer T (NKT) cells*,  *$\gamma\delta$ T cells*, and *nonadaptive natural T regulatory (nTreg) cells*.

From the 12th week of fetal life, thymocyte precursors originate from the lymphoid stem cell and migrate in response to chemokines via the blood from the bone marrow to the thymus. These cells have not yet rearranged their T-cell receptor (TCR) genes, and they lack expression of TCR, accessory antigen receptor molecules, or coreceptors. At the 1st stage, in the subcapsular, or outer cortical zone, the thymocytes are *double-negative (DN) cells*,  $CD4^-CD8^-$ , but the  $\beta$  chain of TCR is expressed. For research purposes, these DN thymocytes are subdivided into DN1, DN2, DN3, and DN4 cells. At the 2nd stage, in the inner cortical zone, thymocytes are *double-positive (DP)*,  $CD4+CD8+$ , and have completed  $\alpha\beta$ TCR. At the 3rd stage, they are *single-positive (SP)*,  $CD4+$  T cells or  $CD8+$  T cells (s. ■ Fig. 1.20).

The thymic microenvironment required for T lymphopoiesis is provided by giant epithelial “nurse” cells, macrophages, thymic dendritic cells, and thymic NK cells. The T lymphopoiesis also depends on many cytokines and chemokines including IL7.

At the DN stage, TCR $\beta$  genes begin rearranging first. At the DP stage, TCR  $\alpha$  chain rearrangement occurs, and both chains are linked together. Gene products of recombination-activating genes (RAG-1 and RAG-2), expressed at all stages of T lymphopoiesis, are necessary both for  $\beta$ -chain and  $\alpha$ -chain rearrangement.

**From a clinical point of view**, mutations in genes of cytokine receptors (IL2RG and IL7RA), some enzymes (AK2 and ADA), signaling molecules (JAK3 and ZAP70), and CD3 chains lead to the fatal disorders of T-cell development and *severe combined immunodeficiency (SCID)* characterized by very severe infections from the birth. In almost all cases, T-cell defects are accompanied by B-cell defects. These conditions required the bone marrow transplantation for children to survive.

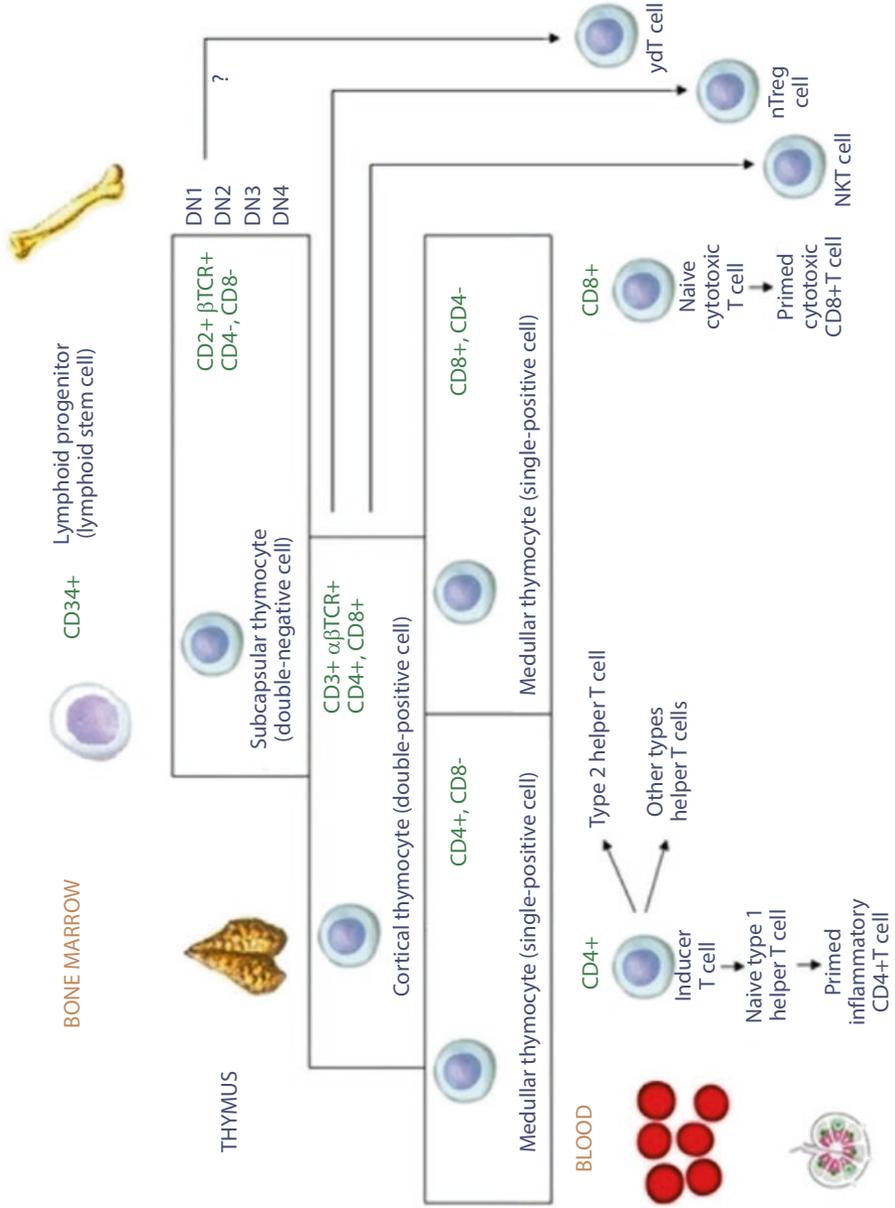


Fig. 1.20 T Lymphopoiesis

It is believed that four major features of T lymphopoiesis or T-cell commitment are available.

1. The *clonal division* into different clones by unique TCR (with a single antigenic specificity), which is linked to an accessory antigen receptor molecule, CD3, and one of two coreceptors, CD4+ or CD8+.
2. Two types of thymic selection: *positive selection*, when TCRs have a low affinity for self-HLA molecules and self-antigens, and *negative selection* (or *clonal deletion*), which induces apoptosis in thymocytes, which bind self-HLA molecules and self-antigens too well or do not bind them at all. However, a small portion of T cells can escape from apoptosis and come into an unresponsive state (*clonal anergy*). Positive selection leads to HLA restriction, and negative selection results in self-tolerance. About 95% of thymocytes cannot escape from negative selection. The gene AIRE upregulates negative selection.
3. The division of all T cells into *two subsets*, naive helper/inflammatory CD4+T cells, and naive cytotoxic CD8+T cells. These subpopulations have different fates.
4. The ability to form *lifelong memory* T cells after naive T cells encounter antigens.

The remaining 5% of mature naive T cells enter the bloodstream and lymph flow to differentiate into helper, effector or memory T cells. They have minimal cytoplasm, condensed chromatin, and little transcriptional activity. A phenotypic marker of naive T cells is CD45RA+. They settle T-dependent zones in the lymph nodes, periarteriolar sheaths in the spleen, and parafollicular zones of MALT. Priming will start out when a T cell encounters an appropriate antigen.

*CD4+T cells* may generate both helper T cells, and effector and memory T cells in the course of CD4+T-cell-mediated immune response. Nowadays, many regulatory and helper CD4+T cells have been described, including the type 1 helper T cell and type 2 helper T cell.

*CD8+T cells* may be either effector or memory T cells in the course of a CD8+T-cell-mediated immune response. This subset typically does not generate regulatory and helper cells.

One more subset, *natural killer T (NKT) cells*, exhibit the functional and phenotypic features of both T cells and NK cells. TCR on the NKT cells can recognize glycolipid antigens presented by the Class I HLA-like molecule CD1d. To date, two types of NKT have been described, including type 1 NKT cells, which express an invariant  $\alpha$ TCR or  $\beta$ TCR and type 2 NKT cells that display noninvariant  $\alpha$ TCR. Being ambivalent cell lineage, they promote tumor-related immunosurveillance or immunosuppression, stimulate or inhibit the development of autoimmune disorders, and promote either inflammation or immune tolerance. Analogous to NK cells, NKT cells enable apoptosis in target cells and contribute to the antiviral defense.

About 0.05% of T cells leaving the thymus are *nonadaptive natural T regulatory (nTreg) cells*. These cells, as well as helper T cells, will be described in ► Sect. 4.9.

In the blood, T cells make up 40–80% of peripheral lymphocytes.

A minor part (0.1–0.5%) of T cells, partially of the thymic development, has  $\gamma\delta$ TCR, and most of these cells express CD8 $\alpha\alpha$ .  $\gamma\delta$ T cells protect against opportunistic microbes in the barrier organs such as epithelial cell-rich compartments like the skin, GI tract, and genitourinary organs (about 50% of the T-cell population). These cells are also called intraepithelial lymphocytes (IELs).  $\gamma\delta$ T cells may be activated by both “patterns” and antigens, being a bridge between the innate and adaptive immunity. During  $\gamma\delta$ TCR gene rearrangement,  $\gamma\delta$ T cells are defined by the inclusion of invariant TCR V-(D)-J segments and are tissue-specific. Therefore, they are characterized by restricted clonal diversity, slower production of effector molecules, and functions similar to NK cell and NKT cell activity.

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

T lymphopoiesis proceeds in:

1. Bone marrow.
2. Spleen.
3. Thymus.
4. Peyer's patches.

#### ? Question 2

Double-negative thymocytes express:

1. CD4+CD8+.
2. CD4–CD8–.
3. CD4+CD8–.
4. CD4–CD8+.

#### ? Question 3

Double-positive thymocytes express:

1. CD4+CD8+.
2. CD4–CD8–.
3. CD4+CD8–.
4. CD4–CD8+.

#### ? Question 4

A keystone cytokine for T lymphopoiesis is:

1. IL7.
2. IL1.
3. IL6.
4. IL21.

#### ? Question 5

Gene products of RAG-1 and RAG-2 are:

1. Tyrosine kinases.
2. Lymphocyte “recombinases.”
3. Adapter proteins.
4. Nuclear transcription factors.

**? Question 6**

$\beta$ TCR is expressed by:

1. Double-positive thymocytes.
2. B cells.
3. Double-negative thymocytes.
4. Single-positive thymocytes.

**? Question 7**

$\alpha\beta$ TCR is expressed by:

1. Double-positive thymocytes.
2. Giant epithelial “nurse” cells.
3. Double-negative thymocytes.
4. Macrophages.

**? Question 8**

During T lymphopoiesis, positive selection means:

1. Apoptosis in thymocytes whose TCRs have a low affinity for self-HLA molecules and self-antigens.
2. Apoptosis of all thymocytes.
3. Clonal deletion.
4. Apoptosis in thymocytes, which bind self-HLA molecules too well and are directed to self-antigens.

**? Question 9**

Naive CD4+ T cells can generate:

1. Helper T cells only.
2. Inflammatory T cells.
3. Effector T cells only.
4. Helper, effector, and memory T cells.

**? Question 10**

Naive CD8+ T cells can generate:

1. Helper T cells only.
2. Cytotoxic and memory T cells.
3. Memory T cells only.
4. Helper, effector, and memory T cells.

**? Question 11**

NKT cells can recognize:

1. Protein antigens.
2. Lipopolysaccharide antigens.
3. Glycolipid antigens.
4. Nothing.

**? Question 12**

Gene AIRE upregulates:

1. Positive selection.
2. Generation of NKT cells.
3. Generation of  $\gamma\delta$ T cells.
4. Negative selection.

**? Question 13**

T cells are able to establish:

1. Short-term memory.
2. No memory.
3. Lifelong memory.
4. B-cell-mediated memory only.

**? Question 14**

Mutation in CD132 can result in:

1. Chronic granulomatous disease.
2. X-linked severe combined immunodeficiency (XSCID).
3. "Lazy Leucocyte" syndrome.
4. Bruton's syndrome.

**? Question 15**

Passed through the positive selection naive T cells settle:

1. T-dependent zones of the secondary lymphoid organs.
2. Follicles of the lymph nodes, spleen, and MALT.
3. Medullary cords of the spleen.
4. The thymus.

**? Question 16**

Intraepithelial lymphocytes (IELs) are:

1. CD4+T cells.
2. NKT cells.
3.  $\gamma\delta$ T cells.
4. Dendritic cells.

### 1.7.3 B Cells and B Lymphopoiesis

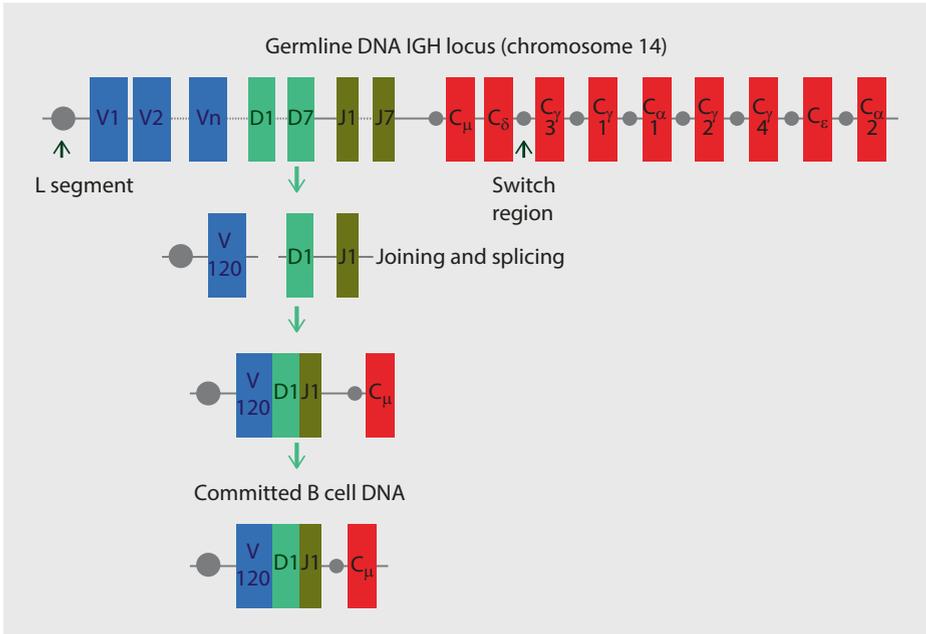
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**Definitions**

*B cells* play a crucial role in the simple and advanced B-cell-mediated immune responses since they have been matured in the bone marrow.

There are *B-1 cells* (CD5+), *B-2 cells*, and *marginal zone B cells* (IgM+IgD+CD1c+CD27+) as well as *natural B regulatory (nBreg) cells*.

During the priming, B cells turn into *plasma cells*.



■ Fig. 1.21 Recombinations in immunoglobulin H chains genes (IGH locus)

From the 10th week of fetal life, B-cell precursors originating from the lymphoid stem cell start out to differentiate into B cells. Early B lymphopoiesis in humans is first induced in the fetus's liver and then within the bone marrow and MALT microenvironment, but it is unknown which cells comprise this niche. If it is to take in simplistic form, there are several stages of the B lymphopoiesis or B-cell commitment.

The germline DNA of B-cell lymphoid precursors (see ■ Fig. 1.21, on a sample of IGH locus) undergoes somatic recombination and splicing that leads to the formation of different DNA complexities in committed B cells. The somatic recombination follows in the specified order: the joining of D and J (at the pro-B-cell stage), random selection of V and formation of VDJ, and addition of C<sub>μ</sub> to VDJ (at the pre-B-cell stage). Splicing enables the cutting of introns (e.g., switch regions and L segment). Subsequently, the pre-B cell undergoes multiple divisions and turns into an immature B cell. L chain rearrangement happens at the immature B-cell stage. Analogous to T lymphopoiesis, gene products, RAG-1 and RAG-2, expressed at stages of B lymphopoiesis, are necessary for both H chain and L chain gene rearrangement.

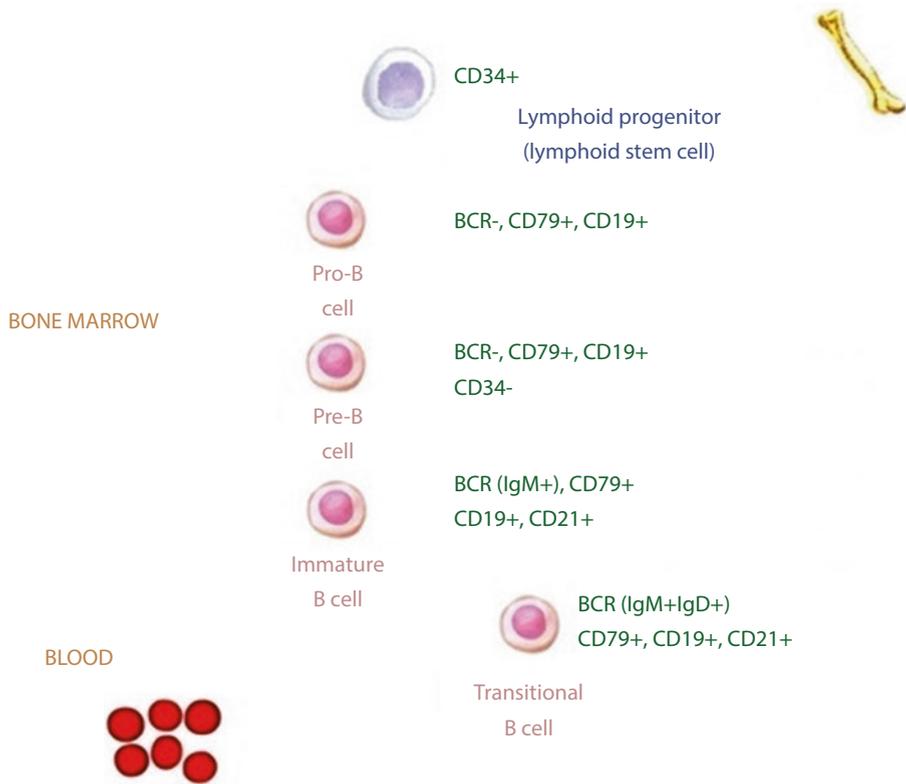
*Pro-B cells* (see ■ Fig. 1.22) already express accessory antigen receptor molecules (Ig $\alpha$  and Ig $\beta$ ), a part of coreceptor (CD19<sup>+</sup>), but not yet BCR. Pro-B cells require direct contact with stromal cells and IL7 secretion to develop.

*Pre-B cells* lose the lymphoid stem cells marker, CD34<sup>-</sup>. The “help” of IL7 is still required though. At this stage, rearrangement of the genes for H chains and L chains is finished.

*Immature B cells* start out to express  $\mu$  chain for BCR (IgM<sup>+</sup>) and one more part of a coreceptor, CD21<sup>+</sup>.

*Transitional B cells* express eventually completed  $\mu\delta$ BCR (IgM+IgD<sup>+</sup>) and migrate to the peripheral blood and lymphoid organs.

It is known that there are four major features of B lymphopoiesis, as follows:



■ Fig. 1.22 B Lymphopoiesis

1. The *clonal division* into different clones by unique BCR (with a single antigenic specificity), which is linked to accessory antigen receptor molecules,  $Ig\alpha$  (CD79a+)/ $Ig\beta$  (CD79b+), and a coreceptor, CD19+/CD21+/CD81+.
2. Two types of B-cell selection: *positive selection*, when BCRs have a low affinity for self-HLA molecules and soluble self-antigens or are edited in V gene region to replace self-antigen directed BCRs by non-self-antigen directed BCRs, and *negative selection*, which induces apoptosis in B cells (*clonal deletion*), which bind self-HLA molecules and cell-associated self-antigens too tightly. However, some B cells escape from apoptosis and come into an unresponsive state (*clonal anergy*). Positive selection leads to HLA restriction, and negative selection results in self-tolerance. About 80% of B cells cannot escape from negative selection.
3. The division of all B cells into *three subsets*, B-1 cells, B-2 cells, and marginal zone B cells. These subpopulations have different activities.
4. The ability to constitute *lifelong memory* B cells after naive B cells encounter antigens in those cases if helper T cells take part in the adaptive immune response.

## 1

*B-1 cells (CD5+)* arise from stem cells during fetal life, have restricted clonal diversity, settle peritoneal and pleural cavities, produce IgM, and do not form memory cells. Analogous to  $\gamma\delta$ T cells, they are a link between the innate and adaptive immunity.

*B-2 cells*, conventional highly diverse cells, are the end cells of an advanced B-cell-mediated immune response to antigens and enable the production of all antibody isotypes and formation of memory B cells. Transitional B cells settle in the T-independent zone of secondary organs of the immune system (lymphoid follicles) and continue their maturation.

*Marginal zone (MZ) B cells (IgM+ IgD+, CD1c+, CD27+)* are located in the spleen at the interface between the circulation and lymphoid tissue. The splenic marginal zone B cells can rapidly respond to blood-borne antigens and “patterns,” being the intermediate subset between B-1 and B-2 subpopulations.

Priming will start out when a B cell encounters an appropriate antigen. Due to differentiation in the course of immune response, the B cell turns into a *plasma cell*, which can produce immunoglobulins.

B cells make up 10–25% of peripheral circulating lymphocytes.

**From a clinical point of view,** mutations in genes of signaling molecules (e.g., BTK), regulatory factors (e.g., RAG-1/RAG-2), costimulatory molecules (e.g., CD40), and chains of B-cell coreceptors (CD19/CD21/CD81) lead to disorders of B-cell development such as *X-linked agammaglobulinemia (Bruton’s syndrome)*, *common variable immunodeficiency (CVID)*, *Hyper-IgM syndrome*, etc. In most cases, they require lifelong intravenous immunoglobulin (IVIG) administration.

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

B lymphopoiesis proceeds in:

1. Fetus’s liver, bone marrow, and MALT.
2. The lymph nodes and spleen.
3. The thymus.
4. Lymph flow and bloodstream.

#### ? Question 2

Gene products of RAG-1 and RAG-2 are:

1. Tyrosine kinases.
2. Lymphocyte “recombinases.”
3. Nuclear transcription factors.
4. Signaling adapter proteins.

#### ? Question 3

Pro-B cells express:

1. BCR.
2. CD21+.
3. CD3+.
4. CD79+.

**? Question 4**

Pre-B cells do not express:

1. CD34+.
2. CD79 $\alpha$ +.
3. CD19+.
4. CD79 $\beta$ +.

**? Question 5**

Immature B cells express:

1. TCR.
2. BCR (IgM+).
3. BCR (IgM+IgD+).
4. CD34+.

**? Question 6**

Transitional B cells express:

1. CD34+.
2. BCR (IgM+).
3. BCR (IgM+IgD+).
4. CD3+.

**? Question 7**

Transitional B cells settle:

1. The thymus.
2. Periarteriolar sheaths of the spleen.
3. T-independent zones of the secondary lymphoid organs.
4. Paracortical zones of the lymph nodes.

**? Question 8**

Conventional highly diverse B-cell subset is:

1. B-2 cells.
2. B-1 cells.
3. Marginal zone B cells.
4. Natural B regulatory cells (nBreg).

**? Question 9**

B cells may turn into:

1. T cells.
2. Plasma cells.
3. Neutrophils.
4. Mast cells.

**? Question 10**

In healthy adults, concentration of B cells in the peripheral blood is about:

1. 0.1–0.5%.
2. 10–25%.
3. 40–80%.
4. 2–5%.

## 1

**? Question 11**

B cells take part in:

1. CD4+T-cell-mediated immune response.
2. B-cell-mediated immune responses.
3. CD8+T-cell-mediated immune response.
4. Phagocytosis.

**? Question 12**

In the course of immune response, B cells may form long-term memory B cells if:

1. Helper CD4+T cells take part in the process.
2. Cytotoxic CD8+T cells participate in the process.
3. Never.
4. In any cases.

**? Question 13**

B cells except natural B regulatory cells (nBreg) are divided into:

1. Two subsets.
2. Four subsets.
3. Three subsets.
4. Many subsets.

**? Question 14**

Clonal anergy of B cells is:

1. Clonal deletion.
2. Unresponsive state.
3. Apoptosis in B cells.
4. Activation of B cells.

**? Question 15**

The somatic recombination of B cell follows in the order:

1. DJ-VDJ-VDJ $C_{\mu}$ .
2. DJ-VDJ $C_{\mu}$ .
3.  $C_{\mu}$ - $C_{\mu}$ DJ- $C_{\mu}$ VDJ.
4. DJ- $C_{\mu}$ DJ-VC $\mu$ DJ.

**? Question 16**

IGH locus is located on:

1. Chromosome 7.
2. Chromosome 2.
3. Chromosome 22.
4. Chromosome 14.

### 1.7.4 Burnet's Clonal Selection Theory

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The 1960 Nobel Laureate Sir Frank Macfarlane Burnet (see  Fig. 1.23) was a great researcher in the history of immunology. To date, his theory is still the accepted explanation for B-cell-mediated adaptive immune responses. Furthermore, the theory may be applied to T-cell-mediated adaptive immune responses too.

■ **Fig. 1.23** Sir  
F. Macfarlane Burnet  
(1899–1985)



1. The “universe of antigens” of the surrounding environment corresponds to the clonal diversity of T cells and B cells in each human body. Nowadays, it is known that most amino acid sequences of TCR and BCR are encoded by the certain genes located on chromosomes 2, 7, 14, and 22.
2. If a random antigen invades the body, the pre-existing T-cell clone and B-cell clone are involved in the adaptive immune responses. These processes result in the formation of specific T cells and antibodies directed to the antigen. Finally, the immune system eliminates the antigen and even develops a memory about this event.
3. Lymphocytic clones specific to self-antigens are deleted, deactivated, or suppressed during T lymphopoiesis and B lymphopoiesis and in the periphery. The state is called self-tolerance.
4. Self-antigens in the *immunoprivileged sites* (the eye, brain, placenta, testes, etc.) are inaccessible for the immune system in the course of T lymphopoiesis and B lymphopoiesis, so there is also self-tolerance to these autoantigens. However, this may be broken down if contact of lymphocytes with these self-antigens occurs.

### 1.7.5 Innate Lymphoid Cells (ILCs)

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#### Definitions

*Innate lymphoid cells (ILCs)* is a collective term for neither T cells nor B cells. ILCs are involved in the innate immunity, inflammation, lymphoid tissue development in fetal life, and immunoregulation. Among recently described ILCs, one ILC subgroup, *NK cells*, has been characterized many years ago.

*Innate lymphoid cells (ILCs)* are recently described immune lymphoid cells that are morphologically similar to T cells and B cells, but lack rearranged antigen-recognizing receptors. ILCs secrete cytokines at high concentrations and are involved in the innate immunity, inflammation, lymphoid tissue development in fetal life, and immunoregulation. ILCs are localized to mucosal surfaces and respond to secreted molecules from the

**Table 1.10** Innate lymphoid cells (ILCs)

Group (signaling)	Cells	Cytokines and mediators	Activity
Group 1 (T-bet)	Thymic NK cells	IFN $\gamma$ (high value)	T lymphopoiesis Defense against viruses, intracellular pathogens, tumors
	NK cells	IFN $\gamma$ (low value), TNF $\alpha$ , perforin, granzymes	Defense against viruses, intracellular pathogens, tumors
	ILC1	IFN $\gamma$	Inflammation Immunoregulation
Group 2 (GATA-3, ROR- $\alpha$ )	ILC2 (nuocytes, natural helper cells)	IL5, IL9, IL13, amphiregulin	Defense against helminths Wound healing Immunoregulation
Group 3 (ROR- $\gamma$ t)	LTi cells (lymphoid tissue inducer cells)	TNF $\beta$ , IL17, IL22	Lymphoid tissue development in the fetal life Intestine homeostasis
Group (signaling)	Cells	Cytokines and mediators	Activity
			Defense against extracellular pathogens
	NCR+ ILC3 (ILC22)	IL22	Epithelium homeostasis Defense against extracellular pathogens Immunoregulation
	NCR- ILC3 (ILC17)	IFN $\gamma$ , IL17	Defense against extracellular pathogens Immunoregulation

epithelium. All ILC populations differentiate from a common lymphoid progenitor, which is located in the fetal liver or adult bone marrow. Because ILCs share developmental and functional similarities with helper T cells, a nomenclature for ILCs has been established based on helper T cells' classification. ILCs are divided into three groups according to the transcription factors mediating their development and the cytokines they secrete (see **Table 1.10**).

*Group-1 ILCs* include NK cells and ILC1 cells. NK cells secrete IFN $\gamma$  and TNF $\alpha$  in response to intracellular pathogens. By means of perforin, granzymes and caspases NK cells trigger the apoptosis in target cells. On the other hand, ILC1 probably function in a synergic manner with type 1 helper T cells. *Group-2 ILCs* produce IL5, IL9, and IL13 in response to helminths. They may function synergistically with type 2 helper T cells. Secreted amphiregulin is a factor, which upregulates the growth of normal epithelial cells. Therefore, ILC2s take part in the resolution of inflammation and support efficient wound healing and tissue repair. Both IL5 and IL13 induce the production of IL4 in

eosinophils that may lead to the thermogenesis. Finally, *group-3 ILCs* are composed of lymphoid tissue inducer (LTi) cells and ILC3 cells that mainly produce IL17 and/or IL22. LTi cells are required for the formation of lymphoid tissues, intestine homeostasis, and defense against extracellular pathogens, whereas ILC3 cells mediate the balance between epithelial symbiotic microbiome and immunity and also take part in defense against extracellular pathogens. The cells of group 3 may function in a synergic manner with type 17 and type 22 helper T cells.

**From a clinical viewpoint,** mutation of CD16 gene is described as *immunodeficiency 20* and characterized by the predisposition to some viral infections (see ► Sect. 3.7). The role of ILC, other than NK cells, is not entirely studied in infections, parasitic invasions, and cancer. However, it expects different ILC may be in an unpredictable manner involved in the promotion, maintenance, or clearance of tumors.

### 1.7.6 Dendritic Cells

#### Definitions

*Dendritic cells (DCs)* are a heterogeneous cell population characterized by outgrowth (dendrite) morphology, high levels of Class I and Class II HLA molecule expression, and qualities of migration, antigen presentation, and activation of naive lymphocytes.

*Dendritic cells (DCs)* are distinguished from the human mononuclear phagocyte system by their outgrowth or dendrite morphology, high levels of Class I and Class II HLA molecules expression, and properties of superior migration, antigen presentation, and activation of naive lymphocytes. Currently, there have been rapid advances in understanding the ontogeny, heterogeneity, and functional specialization of DC subsets in mice, but relatively little is known about the ontogeny, differentiation, and activity of human DC subsets (see ■ Fig. 1.24).

DCs are a heterogeneous cell population taking into account their origin, locations, phenotypes, and immunological functions. This cell type was discovered by 2011 Nobel Laureate R.M. Steinman, but one subset, Langerhans cells, was revealed in 1868 by P. Langerhans.

All DCs are capable of pathogen engulfing, processing, and antigen/Class I or Class II HLA complex presenting to lymphocytes. *Immature DCs* function through the uptake and accumulation of any antigens at the skin and mucosal level, whereas *mature DCs* take part in antigen presentation and initial stages of adaptive immune responses in the secondary lymphoid organs (see ■ Table 1.11). They also can initiate the induction of immunological tolerance. Most described DCs express TLR to recognize “patterns” as well as receptors for cytokines and chemokines and upregulate reactions of the innate immunity.

To date, some functional and morphological subsets of the DCs have been described in humans.

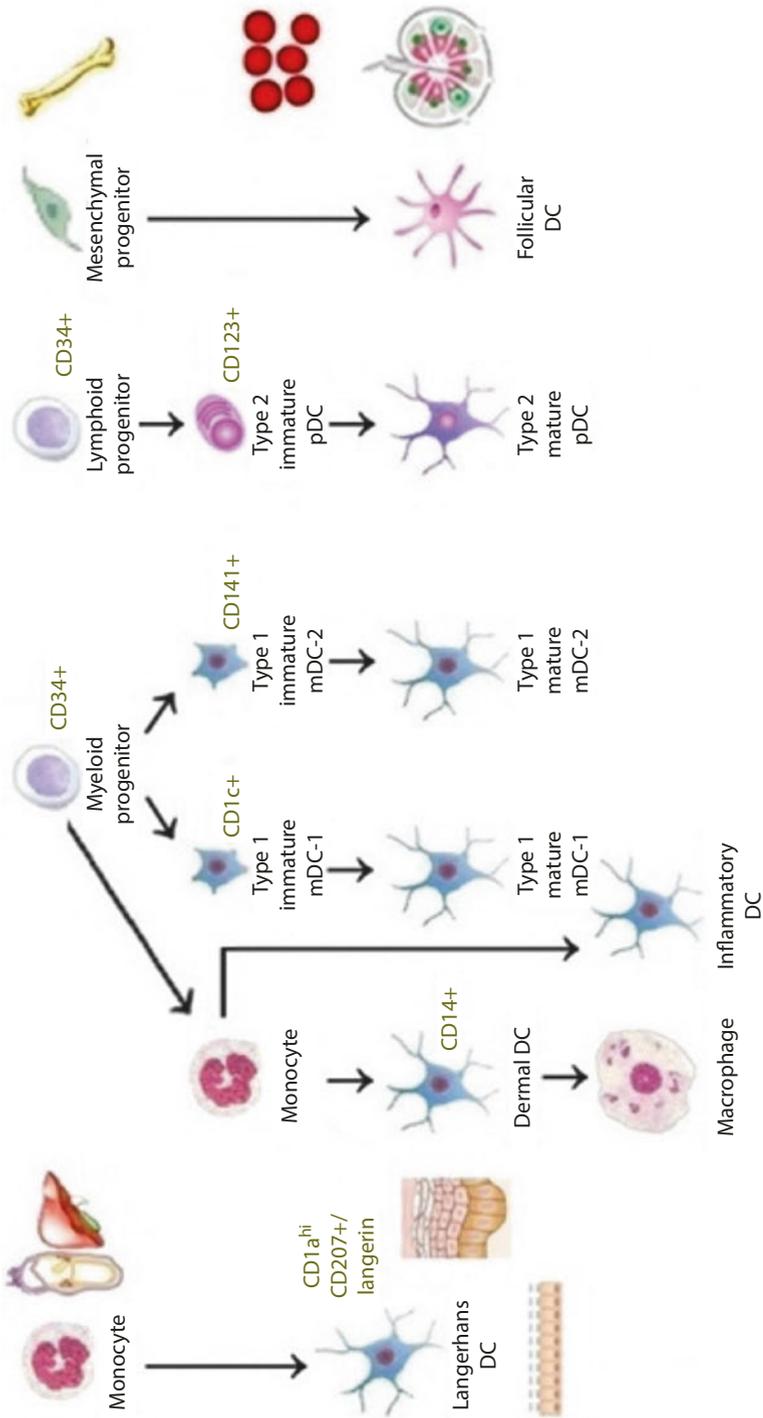


Fig. 1.24 Ontogeny of dendritic cells

■ **Table 1.11** Immature and mature dendritic cells

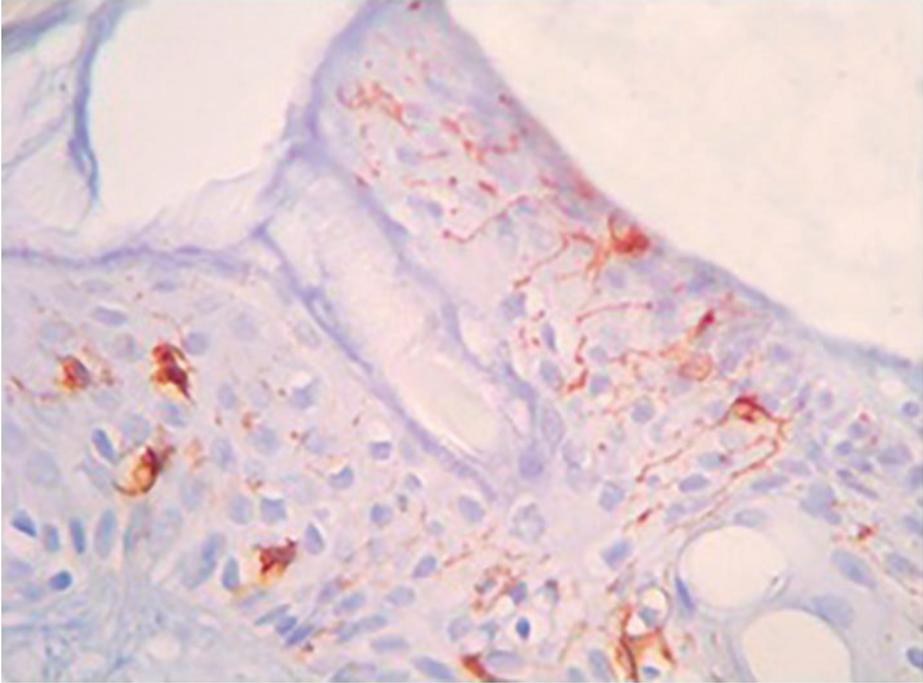
Criterion	Immature	Mature
HLA	(+)	+++ (except follicular DCs)
Costimulatory molecules: CD80, CD86, CD40, etc.	(+)	+++
CD83+ marker	–	++
CCL19/CCR7 CCL20/CCR6 CCL21/CCR6, CCR7	++	–
CCL3/CCR1, CCR5 CCL4/CCR5 CCL5/CCR1, CCR3, CCR5 CXCL1/CXCR1	–	++
Antigen endocytosis	+++	(+)
Antigen presentation	+	+++
Number of dendrites	+	+++
Presence in the blood	0.1–0.5%	–

*Langerhans cells* (see ■ Fig. 1.25) originate from the yolk sac and fetal liver's monocytes. These DCs can be identified by the presence of langerin/CD207-containing Birbeck granules as well as the expression of Class I and Class II HLA molecules and CD1a<sup>hi</sup>. Langerhans cells are located in the epidermis and mucosal epithelium, may uptake antigens, migrate to SALT and MALT-draining lymph nodes, and form *interdigitating cells* to initiate the T-cell-mediated immune responses.

*Dermal DCs*, or *CD14+DCs*, originate from monocytes and are a transient population of monocyte-derived macrophages. Upon inflammation, CD14+ classical monocytes are also the putative precursors of *inflammatory DCs*, which display an activated and pro-inflammatory phenotype.

*Type 1 myeloid (classical or conventional DCs) (mDCs/cDCs)* are myeloid progenitor-derived DCs. They are subdivided into two subtypes: (1) the major DC subset (mDC-1), CD1c+, which mainly expresses Class II HLA molecule and may activate like macrophages naive CD4+T cells to clonal expansion, differentiation, and immune inflammation, and (2) a rarer DC subset (mDC-2), CD141+, which expresses Class I and Class II HLA molecules, is capable of cross-presentation, and may activate both naive CD4+ and CD8+T cells. Both subtypes possess characteristic long outgrowths called dendrites required for antigen presentation and secrete IL12 and other pro-inflammatory cytokines. Mature mDCs are described as *interdigitating DCs*, which are resident in the secondary lymphoid organs. If any mature DCs are located in the barrier organs, they are *interstitial DCs*. *Thymic DCs* in the thymus are closer to interdigitating DCs (mDCs).

*Type 2 plasmacytoid DCs (pDCs)* are lymphoid progenitor-derived DCs that in the immature state are characterized by spherical morphological features similar to plasma



■ Fig. 1.25 Langerhans cells in the epidermis. CD1a+, immunohistochemical staining,  $\times 400$

cells. In the mature state, they acquire conventional DC-like morphology with dendrites, express Class II HLA molecules, CD123+ and CD303+, and secrete IFN $\alpha$  and IFN $\beta$ . It is probable that pDCs are involved in the B-cell-mediated response and antiviral defense.

Most described DCs express TLR to recognize “patterns” as well as receptors for cytokines and chemokines and upregulate reactions of the innate immunity.

*Follicular DCs (fDCs)*, a distinct subset, appear to arise from mesenchymal progenitor cells. They are located in lymphoid follicles of the lymph nodes, spleen, and MALT and have dendrites and high expression of complement receptors CR1 (CD35), CR2 (CD21), and Fc $\gamma$ RIIb (CD32) that allow them to serve both as a depository for antigens and as a source of the continued activation for the B-cell-mediated response. The major fDC function is to bind and retain antigens by linking to complement and immune complexes and then present these antigens to germinal center B cells that drive the secondary immune response. However, fDCs never express HLA molecules and cannot initiate the primary immune response.

*Veiled DCs* are a morphological type of most DCs, intermediate between immature and mature DCs, which are found in the lymph flow.

**From a clinical viewpoint,** there is a great interest in using DCs to develop immunotherapies for cancer, autoimmune disorders, recurrent infections, and induction of graft tolerance utilizing dendritic cell-based vaccines (see ► Chap. 8).

**■ Quiz**

Reading a question, please choose only one right answer.

**? Question 1**

Follicular DCs generate from:

1. Myeloid progenitor.
2. The yolk sac.
3. Mesenchymal progenitor.
4. Lymphoid progenitor.

**? Question 2**

Type 1 myeloid DCs are divided into:

1. mDC-1 and mDC-2.
2. pDC and fDC.
3. Langerhans DC and dermal DC.
4. Some inflammatory DCs.

**? Question 3**

Inflammatory DCs may originate from:

1. Langerhans DC.
2. Follicular DC.
3. Lymphoid progenitor.
4. Monocyte.

**? Question 4**

Mature DCs have:

1. Many dendrites.
2. A few dendrites.
3. No dendrites.
4. Large granules.

**? Question 5**

Immature DCs are mainly located in:

1. The thymus.
2. Barrier organs.
3. The bone marrow.
4. The kidney.

**? Question 6**

HLA molecules are mainly expressed on:

1. Immature DCs.
2. Lymphoid progenitors.
3. Most of mature DCs.
4. Follicular DCs.

**? Question 7**

Dermal DCs are:

1. fDCs.
2. Type 2 mature pDCs.
3. A transient population of monocyte-derived macrophages.
4. Type 1 mature mDCs.

**? Question 8**

Langerhans DCs generate from:

1. Macrophages.
2. Mesenchymal progenitors.
3. The yolk sac and fetal liver's monocytes.
4. Lymphoid progenitors.

**? Question 9**

Type 2 plasmacytoid DCs secrete:

1. IL12.
2. IFN $\alpha$  and IFN $\beta$ .
3. Somatotropin.
4. Aldosterone.

**? Question 10**

Birbeck granules are located in:

1. Type 2 immature pDCs.
2. Langerhans DCs.
3. Type 1 immature mDCs.
4. Macrophages.

**? Question 11**

Langerhans DCs and type 1 mDCs can form:

1. Follicular DCs.
2. Type 2 pDCs.
3. Interdigitating DCs.
4. Macrophages.

**? Question 12**

Interstitial DCs are:

1. Mature DCs of barrier organs.
2. Type 1 immature mDCs-1.
3. Type 2 immature pDCs.
4. Type 1 immature mDCs-2.

**? Question 13**

Type 1 myeloid DCs secrete:

1. Glucocorticoids.
2. IL35.
3. IL12.
4. IFN $\alpha$  and IFN $\beta$ .

**Question 14**

DCs as a cell type were discovered by:

1. F.M. Burnet.
2. R.M. Steinman.
3. E.E. Metchnikoff.
4. P. Ehrlich.

**Question 15**

DCs are functionally related to:

1. Innate and adaptive immunity.
2. Adaptive immunity only.
3. Innate immunity only.
4. Neurons of the CNS.

**Question 16**

DCs, which serve both as a depository for antigens and as a source of the continued activation of B cells, are:

1. Type 1 mDC.
2. Type 2 pDCs.
3. Langerhans DCs.
4. fDCs.

## 1.7.7 Monocytes and Macrophages

### Definitions

*Monocytes* are related to WBC and have a bean-shaped nucleus. Monocytes are precursors of some macrophages, dermal DCs, and Langerhans DCs.

*Macrophages* are large mononuclear cells important for both innate and adaptive immunity. They are able to *phagocyte* large objects like protozoans and infected cells, *secrete* a lot of active substances like cytokines, fulfill the *presentation* of antigen/Class II HLA molecules complexes to CD4+ T lymphocytes, and take part in *type IV hypersensitivity*.

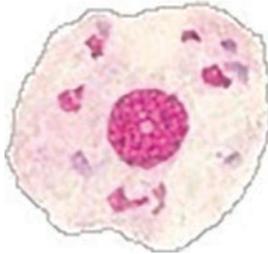
There are two distinct types of macrophages: (1) *inflammatory (M1)* and (2) *anti-inflammatory (M2)*. Also, *tumor-associated macrophages (TAMs)* characterized by ambivalent patterns of activity are described.

*Monocytes* (see ■ Fig. 1.26) originate from hematopoietic stem cells in the bone marrow. Normally, monocytes make up about 6–8% of white blood cells (WBC). They may circulate in the blood for about 24 h and then differentiate into a type of macrophages (see ■ Fig. 1.27), *inflammatory macrophages (M1)*, which are important during pathogen invasions and tissue injury. They are activated by IFN $\gamma$  and lipopolysaccharide (LPS). M1 macrophages are antigen-presenting cells for CD4+T cell-mediated immune response. Another type of macrophages is known, *anti-inflammatory macrophages (M2)*, which are activated by IL4 and IL13 and secrete IL10 and TGF $\beta$ . They play a role in constructive processes such as wound healing and tissue repair but also in tumor

■ Fig. 1.26 Monocyte



■ Fig. 1.27 Macrophage

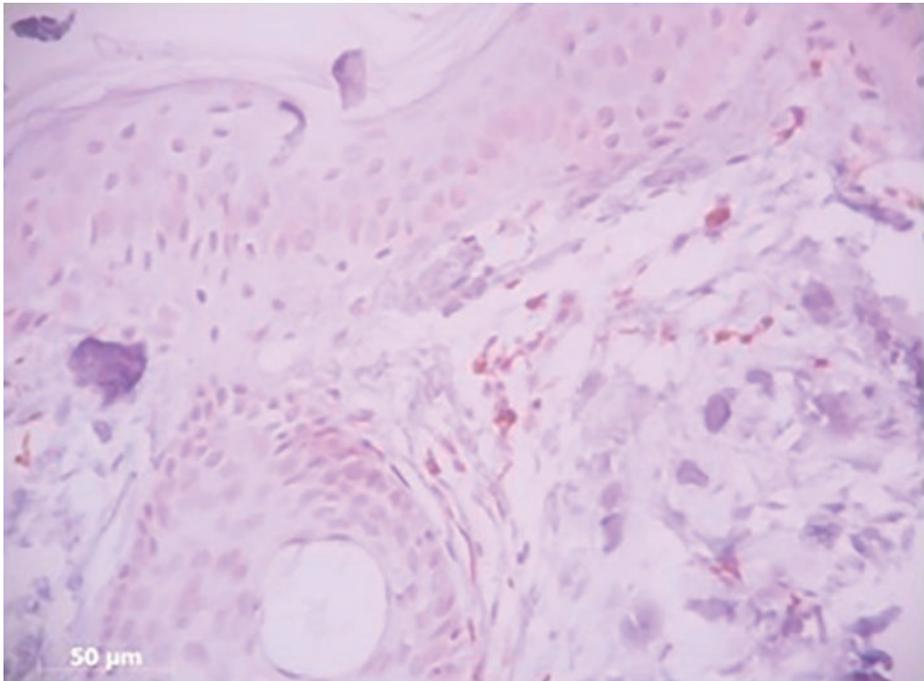


growth. Macrophages were first discovered by 1908 Nobel Laureate E.E. Metchnikoff who also first discovered phagocytosis. Chemokines responsible for monocyte trafficking are CCL7 (MCP-3) and CX3CL1 (Fractalkine). Chemokines involved in macrophage migration are CXCL14 (BRAK), CCL3 (MIP-1 $\alpha$ ), CCL4 (MIP-1 $\beta$ ), and CCL5 (RANTES).

In the absence of infections, all tissues of the body contain *tissue-resident macrophages* or tissue-specialized types of macrophages that are available throughout human life:

- Intestinal lamina propria macrophages (GI tract)
- Peritoneal macrophages (the peritoneal cavity)
- Kupffer's cells (the liver)
- Intraglomerular mesangial cells (the kidney)
- Alveolar macrophages (the lungs)
- Pleural macrophages (the pleural cavity)
- Dermal macrophages (the skin)
- Sinus histiocytes (the lymph nodes)
- Splenic macrophages (marginal zone, red and white pulp of the spleen)
- Osteoclasts (the bone)
- Microglial, perivascular, and meningeal macrophages (the CNS)

They play roles in tissue development, muscle regeneration, clearance of dead cells, and cellular debris (like scavenger cells) and may take part in antigen processing and presentation, immune inflammation, and interaction with tumors. It is the well-known plasticity of macrophages that allows them to change their behavior depending on distinct signals. They may be activated to kill intracellular pathogens (M1) and, alternatively, may limit inflammation and stimulate wound healing (M2). *Tumor-associated macrophages*



■ Fig. 1.28 Macrophages in the dermis. CD68+, immunohistochemical staining,  $\times 400$

(TAMs) best demonstrate the ambivalence of this cell lineage because they may be involved in both pro-tumor and antitumor processes.

CD68 (see ■ Fig. 1.28), CD40, Fc $\gamma$ RI (CD64), Toll-like receptors (TLRs), scavenger receptors (CD163, CD206, and CD209), cytokine, and chemokine receptors are common macrophage markers. Macrophages secrete an enormous number of various biologically active molecules: cytokines, chemokines, enzymes including metalloproteinases (e.g., collagenase), complement proteins, reactive oxygen species (ROS) including NO $^-$ , arachidonic acid's metabolites (prostaglandins and leukotrienes), etc. The profile of cytokines depends on the M1 type or M2 type of the cells. M1 macrophages secrete IL12, TNF $\alpha$ , IFN $\gamma$ , IL1, IL6, IL15, IL18, and IL23, while M2 macrophages produce IL10 and TGF $\beta$ .

**From a clinical point of view,** in some cases, some pathogens can subvert the phagocytosis fulfilled by macrophages and hide inside these cells from the immune system. It is so-called uncompleted phagocytosis. As a consequence, the pathogens can replicate and become more dangerous for the body in the future. Such pathogens are *HIV*, *Mycobacteria*, *Leishmania*, *Brucella*, *Legionella*, etc.

On the other hand, macrophages may contribute to cancer growth and progression and take part in creating the atherosclerosis plaque lesions and obesity complications like type 2 diabetes mellitus.

## 1

## ■ Quiz

Reading a question, please choose only one right answer.

## ? Question 1

Monocytes circulate in the blood for about:

1. 72 h.
2. 24 h.
3. 1 h.
4. 30 min.

## ? Question 2

Monocytes may turn into:

1. Langerhans DCs.
2. Neutrophils.
3. Lymphocytes.
4. Hepatocytes.

## ? Question 3

Macrophages were first discovered by:

1. P. Ehrlich.
2. L. Pasteur.
3. F.M. Burnet.
4. E.E. Metchnikoff.

## ? Question 4

M1 are important for:

1. CD4+T-cell-mediated immune response.
2. CD8+T-cell-mediated immune response.
3. Simple B-cell-mediated immune response.
4. Advanced B-cell-mediated immune response.

## ? Question 5

These cells are related to the tissue-resident macrophages:

1. Hepatocytes.
2. Osteoclasts.
3. Neurons.
4. Monocytes.

## ? Question 6

M2 can be activated by:

1. IL1.
2. IL8.
3. IL13.
4. IFN $\gamma$ .

**? Question 7**

A type of macrophages, which can upregulate wound healing and tissue repair, is:

1. M1.
2. mDC.
3. M2.
4. pDC.

**? Question 8**

A type of macrophages, which can kill intracellular pathogens, is:

1. M1.
2. M2.
3. mDC-1.
4. mDC-2.

**? Question 9**

Macrophage, which can be involved in both pro-tumor and antitumor processes, is:

1. M1.
2. Osteoclast.
3. TAM.
4. Kupffer's cell.

**? Question 10**

M1 secrete:

1. IL35.
2. TNF $\alpha$ .
3. IL10.
4. TGF $\beta$ .

**? Question 11**

M2 secrete:

1. IL12.
2. IL1.
3. IL18.
4. IL10.

**? Question 12**

Phagocytosis by macrophages may result in:

1. Uncomplete phagocytosis.
2. NETosis.
3. Apoptosis in those macrophages.
4. B-cell-mediated responses.

**? Question 13**

Kupffer's cells are:

1. Circulating macrophages.
2. Circulating monocytes.
3. Tissue-resident macrophages in the liver.
4. Microglial macrophages in the CNS.

1

**Question 14**

This molecule is a macrophage's marker:

1. CD4.
2. CD68.
3. CD8.
4. CD19.

**Question 15**

Macrophages can produce reactive oxygen species (ROS):

1. Yes.
2. No.
3. Nitric oxide only.
4. Never.

**Question 16**

TGF $\beta$  is secreted by these types of macrophages:

1. Monocyte.
2. Langerhans DC.
3. M1.
4. M2.

## 1.7.8 Neutrophils

---

### Definitions

*Neutrophils* are the major cell lineage of WBC in the peripheral blood in healthy adult persons. They have a segmented nucleus and three types of granules containing microbicidal factors. There are two pools of neutrophils, a *circulating pool* and *marginal pool*. Neutrophils are capable of *phagocytosing* and destroying extracellular pathogens like bacteria and molds. Also, neutrophils can fulfill *NETosis* to attack pathogens in a specific manner.

*Neutrophils* (see ■ Fig. 1.29) and neutrophil activity may be a crucial even urgent factor in the innate immunity. They migrate very fast toward the site of infection, fight the infection by means of *phagocytosis* and *netosis* (*NETosis*), and become *kamikaze cells* because they all undergo autolysis during their activity. Therefore, neutrophils are short-lived cells and survive no more than 1–2 days.

There are two pools of neutrophils, a *circulating pool* and *marginal pool*. Cells of the marginal pool are distributed close to the vascular endothelium. Circulating neutrophils are a major cell type among WBC, making up 45–75%. However, in healthy children from 4–5 days old to 4–5 years of age, lymphocytes predominate over neutrophils in the bloodstream.

Neutrophils generate from stem cells in the bone marrow in response to IL3, GM-CSF, and G-CSF. Early blood forms are called banded neutrophils, while mature cells look like segmented neutrophils with 3–5 segmented nuclei. The cytoplasm of the cells

■ Fig. 1.29 Neutrophil



■ Table 1.12 Neutrophil's granules

Granule type	Antimicrobial/inflammatory factors
Azurophilic (primary)	Myeloperoxidase, $\alpha$ defensins, neutrophil elastase, bactericidal/permeability-increasing protein
Specific (secondary)	NADPH oxidase, lysozyme, lactoferrin, cathelicidin, histaminases
Tertiary	Metalloproteinases (collagenase, gelatinase)

contains 200 granules of three types (see ■ Table 1.12), larger azurophilic (1/3), smaller specific (2/3), and tertiary (very few), which are degranulated upon phagocytosis.

A wide range of cytokines and chemokines regulate neutrophil activity including *IL8* (*CXCL8*), *IL17*, *CXCL1* (*GRO $\alpha$* ), *CXCL2* (*MIP-2 $\alpha$* , *GRO $\beta$* ), *CXCL3* (*MIP-2 $\beta$* , *GRO $\gamma$* ), *CXCL5* (*ENA-78*), *CXCL6* (*GCP-2*), and *CXCL7* (*NAP-2*).

Analogous to TAMs, *tumor-associated neutrophils* (*TANs*) are recently described as a neutrophil population different from conventional neutrophils and granulocyte fraction of myeloid-derived suppressor cells (*MDSCs*), and it is a fertile field of the current research.

**From a clinical point of view**, there are some terms, which serve as conventional markers for monitoring many pathological conditions. *Leukocytosis* means an increase in neutrophils and other WBC. As a rule, leukocytosis is present in a systemic or even severe local inflammation. Leukocytosis (neutrophilosis) may result from a shift of WBC from the marginal pool to the circulating pool or due to the flow of WBC from the bone marrow. Sometimes, leukocytosis may be an alarm sign of a hyperleukemic form of leukemia.

*Leukopenia* means a decrease in WBC including neutrophils. The terms “leukopenia” and “neutropenia,” a subtype of leukopenia, may sometimes be used interchangeably. Leukopenia may appear under many clinical conditions such as chemotherapy, radiation exposure conditions, a leukopenic form of the leukemia, hypoplastic anemia, *HIV/AIDS*, systemic lupus erythematosus (*SLE*), etc.

Leukopenia should not be confused with *agranulocytosis*, a more severe lack of one predominant type of infection-fighting WBC. Agranulocytosis may lead to the life-threatening condition of suppressed innate immunity.

About *primary immunodeficiencies*, which are associated to neutrophils, see ► Sect. 3.5.

### 1.7.9 Eosinophils

#### Definitions

*Eosinophils* are the cell lineage of WBC important for defense against parasitic invasions. In addition, they take part in *type I hypersensitivity* and so-called eosinophil inflammation. Analogous to neutrophils, eosinophils are able to *phagocytosis* and *NETosis*.

*Eosinophils* (see ■ Fig. 1.30) originate from stem cells and mature in the bone marrow in response to IL3, GM-CSF, and IL5. In healthy persons, eosinophils make up about 2–5% of WBC, circulate about 8–12 h, and can survive up to 8–12 days. They are part of the innate immunity and protect against helminth parasites and some protozoans. They also take part in so-called eosinophil inflammation during the late phase of type I hypersensitivity.

Eosinophils have small cytoplasmic granules, which contain major basic protein, eosinophilic peroxidase, enzymes, histaminase, etc. Most of these factors may be released during degranulation, affect parasites in a toxic manner, and, as a rule, participate in inflammatory processes. Analogous to neutrophils, eosinophils are capable of phagocytosis and NETosis.

Eosinophils can attack parasites like *Schistosoma larvae* through antibody-dependent cell-mediated cytotoxicity (ADCC) by IgE. First, IgE antibodies coat these pathogens and then are bound to FcεRII (CD32) on eosinophils, which affect parasites by means of eosinophil toxic factors. This mechanism is related to the adaptive immunity.

Some chemokines such as CCL11 (Eotaxin-1), CCL24 (Eotaxin-2), and CCL26 (Eotaxin-3) play a role in the eosinophil migration and other patterns of their activity. However, *IL5* is the major cytokine that upregulates most processes associated with eosinophils.

**From a clinical viewpoint,** *eosinophilia*, i.e., elevated eosinophil count in the blood, may be caused by parasitic invasions, allergic conditions such as atopic dermatitis, allergic rhinitis, asthma, drug allergy, forms of primary immunodeficiencies called *Hyper-IgE syndrome* (dominant form, or Job's syndrome, caused by mutation in STAT3 on 17q21.2, and recessive form, caused by mutation in DOCK8 on 9p24.3), as well as autoimmune/endocrine disorders, systemic mastocytosis, eosinophilic esophagitis, and some types of tumors or be idiopathic.

■ Fig. 1.30 Eosinophil



An *eosinophilic leukemoid reaction* means an increase in the eosinophil count in the blood of non-leukemia origin. It may be caused by a massive parasitic invasion, individual drug allergic condition, bullous pemphigoid, intrahepatic cholestasis, arterial thrombosis, etc. In any case, real leukemia may be excluded by the trepanobiopsy, a tool for the study of bone marrow's cells in flow cytometry or immunohistochemical staining.

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

A pool of neutrophils distributed close to vascular endothelium is:

1. Bone marrow's.
2. Marginal.
3. Circulating.
4. Skin's.

#### ? Question 2

Early blood forms of neutrophils are:

1. Cells with bean-shaped nucleus.
2. Banded neutrophils.
3. Segmented neutrophils.
4. Veiled DCs.

#### ? Question 3

Myeloperoxidase is present in:

1. Specific granules of neutrophils.
2. Secondary granules.
3. Tertiary granules.
4. Azurophilic granules.

#### ? Question 4

Neutrophils are able to:

1. Phagocytosis only.
2. NETosis only.
3. Phagocytosis and NETosis.
4. Antigen processing.

#### ? Question 5

Lysozyme is present in:

1. Primary granules of neutrophils.
2. Secondary granules.
3. Tertiary granules.
4. Azurophilic granules.

**? Question 6**

Lactoferrin is present in:

1. Primary granules of neutrophils.
2. Secondary granules.
3. Tertiary granules.
4. Azurophilic granules.

**? Question 7**

“Leukocytosis” means:

1. A decrease in WBC number including neutrophils in the blood.
2. Severe lack of neutrophils.
3. An increase in WBC number including neutrophils.
4. Leukopenia.

**? Question 8**

The major cytokine/chemokine that upregulates the neutrophil activity is:

1. IL8 (CXCL8).
2. CXCL12 (SCDF-1).
3. CCL25 (TECK).
4. IL10.

**? Question 9**

“Agranulocytosis” means:

1. An increase in WBC number including neutrophils.
2. Severe lack of neutrophils.
3. Leukopenia.
4. Hypoplastic anemia.

**? Question 10**

The major cytokine that upregulates the eosinophil activity is:

1. IL1.
2. IL5.
3. IL35.
4. IL17.

**? Question 11**

Eosinophils circulate in the bloodstream about:

1. 1 month.
2. 15 days.
3. 8–12 h.
4. 1 year.

**? Question 12**

Eosinophils may survive up to:

1. 8–12 days.
2. 1 year.

3. 12 months.
4. 30 days.

**Question 13**

Eosinophils are important for defense against:

1. *Mycobacterium tuberculosis*.
2. HIV.
3. *Candida albicans*.
4. Helminth parasites.

**Question 14**

Analogous to NK cells, eosinophils can develop:

1. Antiviral activity.
2. Antibody-dependent cell-mediated cytotoxicity (ADCC).
3. Efficient antitumor activity.
4. Activation along with cytotoxic CD8+T cells.

**Question 15**

"Eosinophilic leukemoid reaction means:

1. A decrease in the eosinophil count in the blood.
2. An increase in the eosinophil count in the blood due to leukemia.
3. An increase in the eosinophil count in the blood of non-leukemia origin.
4. Common eosinophilia.

**Question 16**

Chemokines important for eosinophil migration are:

1. CXCL1 (GRO $\alpha$ ), CXCL2 (MIP-2 $\alpha$ , GRO $\beta$ ), and CXCL3 (MIP-2 $\beta$ , GRO $\gamma$ ).
2. CCL7 (MCP-3) and CX3CL1 (Fractalkine).
3. CCL3 (MIP-1 $\alpha$ ), CCL4 (MIP-1 $\beta$ ) and CCL5 (RANTES).
4. CCL11 (Eotaxin-1), CCL24 (Eotaxin-2), and CCL26 (Eotaxin-3).

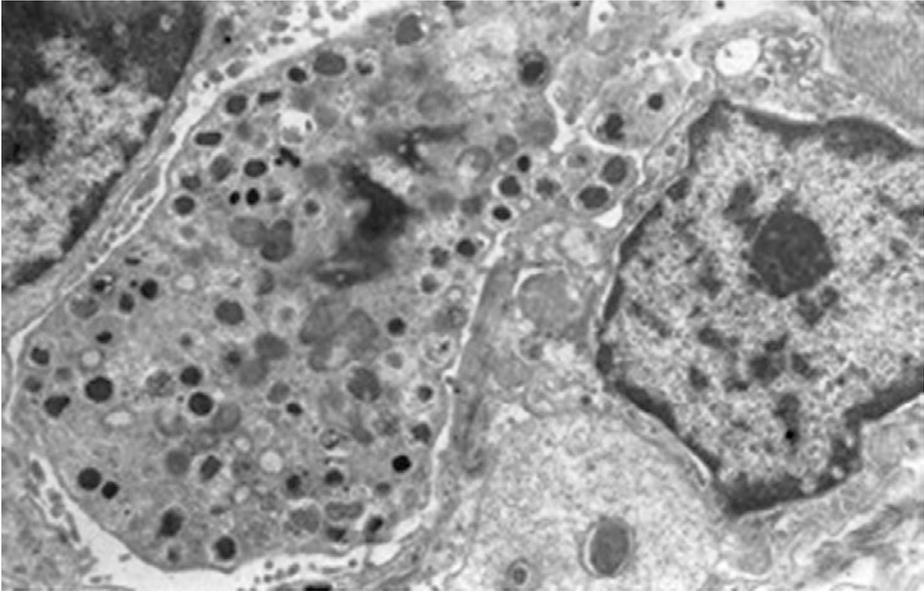
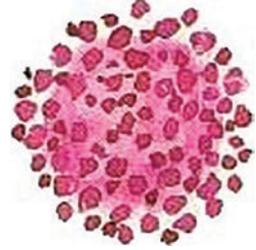
## 1.7.10 Basophils and Mast Cells

### Definitions

*Basophils* are the minor cell lineage of WBC containing lots of large basophilic granules and function similar to mast cells.

*Mast cells* are large cells found in connective tissues throughout the body, most abundantly in the skin, submucosa (a connective tissue phenotype), and mucosa (a mucosal or atypical phenotype). Mast cells contain many large granules rich in histamine, chemotactic peptides, arachidonates, proteoglycans, etc. High-affinity Fc $\epsilon$ RI receptors (Fc $\epsilon$ RI) allow mast cells to bind IgE molecules that leads to their degranulation and activation at early phase of *type 1 hypersensitivity*. Analogous to neutrophils and eosinophils, mast cells are able to develop *phagocytosis* and *NETosis*.

■ Fig. 1.31 Mast cell



■ Fig. 1.32 Connective tissue mast cell in the skin, electronic microphotograph,  $\times 7200$

*Basophils* and *mast cells*, or tissue basophils (see ■ Fig. 1.31), originate from a stem cell in the bone marrow. However, basophils migrate to the blood when mature, whereas the mast cells first circulate in the immature form, then they are scattered throughout connective tissue close to blood vessels and lymphatics and undergo their subsequent differentiation. Mast cells were first described by 1908 Nobel Laureate P. Ehrlich. Analogous to basophils, mast cells have many (50–200) large granules, which are rich in the following:

1. *Preformed mediators* such as histamine, serotonin, chemotactic peptides for neutrophils and eosinophils, enzymes, and heparin
2. *Newly formed mediators* such as thromboxane, leukotriene C<sub>4</sub> (LTC<sub>4</sub>), leukotriene B<sub>4</sub> (LTB<sub>4</sub>), prostaglandin D<sub>2</sub> (PGD<sub>2</sub>), and platelet-activating factor (PAF)

There are two phenotypes of mast cells, *connective tissue mast cells* (see ■ Fig. 1.32) and *mucosal (atypical) mast cells* (see ■ Table 1.13).

■ **Table 1.13** Phenotypes of mast cells

Feature	Connective tissue mast cells	Mucosal (atypical) mast cells
Tryptase	+	+
Chymase	+	–
Histamine	Much	Less
Prevalent proteoglycan	Heparin	Chondroitin sulfate
Prevalent arachidonate	PGD2	LTC4
Size	Larger	Smaller
Prevalent localization	Skin, submucosa	Mucosa
Dependency on T cells	–	+

Mast cells are upregulated by type 9 helper T cells (IL9, IL10), type 2 helper T cells (IL4), and some chemokines including CCL11 (Eotaxin-1), CCL24 (Eotaxin-2), and CCL26 (Eotaxin-3). On the other hand, mast cells play a role in the differentiation of type 2 helper T cells. It is well-known that mast cells play a significant role during the early phase reaction of type I hypersensitivity under such conditions as atopic allergic diseases and anaphylactic shock. A major receptor of mast cells through which they take part in type I hypersensitivity is FcεRI.

Analogous to neutrophils and eosinophils, mast cells can also phagocyte and fulfill NETosis. Besides, mast cells are involved in defense against parasites, wound healing, maintenance of the blood-brain barrier, fertility, and angiogenesis. Surprisingly, considerable progress has been made in understanding that mast cells belong to both the innate and adaptive immunity because they can function as antigen-processing cells and cells, which present antigen/HLA molecules to lymphocytes.

**From a clinical viewpoint,** it is significant *allergic skin tests* are based on the determination of allergen-specific IgE antibodies, which are bound to FcεR on mast cells. Also, a hives-like immunopathology that involves mast cells and may be diagnosed using *tryptase* is *systemic mastocytosis*.

#### ■ Quiz

Reading a question, please choose only one right answer.

#### ❓ Question 1

Basophils originate from:

1. The yolk sac.
2. The spleen.
3. The bone marrow.
4. The thymus.

## 1

**? Question 2**

Mast cells were first discovered by:

1. P. Ehrlich.
2. EE. Metchnikoff.
3. F.M. Burnet.
4. P. Langerhans.

**? Question 3**

Preformed mediators of mast cell's granules are:

1. Leukotrienes LTC<sub>4</sub> and LTB<sub>4</sub>.
2. Prostaglandin D<sub>2</sub> (PGD<sub>2</sub>).
3. Eosinophilic peroxidase.
4. Histamine and heparin.

**? Question 4**

Newly formed mediators of mast cell's granules are:

1. Leukotrienes LTC<sub>4</sub> and LTB<sub>4</sub>.
2. Histamine and heparin.
3. Chemotactic peptides for neutrophils and eosinophils.
4. Lactoferrin.

**? Question 5**

Mast cells are important for:

1. Late phase of type I hypersensitivity.
2. Early phase of type I hypersensitivity.
3. Type III hypersensitivity.
4. Type IV hypersensitivity.

**? Question 6**

Connective tissue mast cell's phenotype is characterized by:

1. Dependency on T cells.
2. Smaller size.
3. The presence of chymase.
4. The prevalence of LTC<sub>4</sub> as compared to PGD<sub>2</sub>.

**? Question 7**

Characteristic receptor on mast cells is:

1. Fc $\delta$ R.
2. Fc $\mu$ R.
3. Fc $\epsilon$ RI.
4. Fc $\epsilon$ RII (CD32).

**? Question 8**

Probably, mast cells may participate in antigen processing:

1. Probably.
2. Never.

3. No.
4. Unknown.

**?** Question 9

As predominant, mast cells of mucosal (atypical) phenotype are located in:

1. The liver.
2. Mucosa.
3. Skin.
4. Submucosa.

**?** Question 10

Mast cells are upregulated by:

1. Type 1 helper T (Th1) cells.
2. Type 9 helper T (Th9) cells.
3. Type 17 helper T (Th17) cells.
4. Follicular helper T (Tfh) cells.

**?** Question 11

Chemokines important for mast cell migration are:

1. CXCL1 (GRO $\alpha$ ), CXCL2 (MIP-2 $\alpha$ , GRO $\beta$ ), and CXCL3 (MIP-2 $\beta$ , GRO $\gamma$ ).
2. CCL7 (MCP-3) and CX3CL1 (Fractalkine).
3. CCL3 (MIP-1 $\alpha$ ), CCL4 (MIP-1 $\beta$ ) and CCL5 (RANTES).
4. CCL11 (Eotaxin-1), CCL24 (Eotaxin-2), and CCL26 (Eotaxin-3).

**?** Question 12

IL4 can affect mast cells:

1. Yes.
2. Probably.
3. No.
4. Never.

**?** Question 13

Mast cells are able to NETosis:

1. No.
2. Never.
3. Probably.
4. Yes.

**?** Question 14

Mast cells are able to phagocytosis:

1. Never.
2. Yes.
3. No.
4. Probably.

**? Question 15**

Mast cells can be involved in maintenance of the blood-brain barrier:

1. Yes.
2. No.
3. Never.
4. Unknown.

**? Question 16**

Probably, mast cells may take part in the presentation of antigen/HLA molecules complexes to lymphocytes:

1. Unknown.
2. Never.
3. No.
4. Probably.

**Key Points**

1. During the ongoing war between “self” (the human body), “non-self” (pathogens), and “former self” (tumors), the immune system uses two main mechanisms, innate immunity with no memory about the battle events and adaptive immunity, which includes the formation of long-term memory about the conflicts. However, the end effects of the immunological mechanisms may be both a full immune clearance and a temporary immune containment.
2. For innate and adaptive immunity, the subjects of immune sensing are “molecular patterns” and antigens.
3. The immune system is well-hierarchized in the ensemble of organs, cells, and molecules. Key components of the system are the thymus, bone marrow, lymphatic network, T cells and B cells with their antigen-recognizing receptors, and cytokines and chemokines. However, immune processes always require the involvement of a variety of other cells and molecules including dendritic cells, phagocytes, mast cells, innate lymphoid cells (ILCs), pattern recognition receptors (PRRs), cell adhesion molecules, etc.

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# Skin and Mucosal Immune System

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**Electronic supplementary material** The online version of this chapter ([https://doi.org/10.1007/978-3-030-03323-1\\_2](https://doi.org/10.1007/978-3-030-03323-1_2)) contains supplementary material, which is available to authorized users.

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## Learning Objectives

*Knowledge.* Upon successful completion of the chapter, students should be able to:

1. Describe the compartments of mucosae-associated lymphoid tissue (MALT), involved cells, and chemokines.
2. Explain how antimicrobial factors including sIgA act at the local level of the immune system.
3. Distinguish between the different compartments of MALT.

*Acquired Skills.* Upon successful completion of the chapter, students should demonstrate the following skills, including:

1. Interpret the knowledge related to skin and mucosal level of the immune system.
2. Critically evaluate the scientific literature about the structure and functions of MALT.
3. Discuss the scientific articles from the current research literature to criticize experimental data and formulation of new hypotheses in basic immunology.
4. Attain a clear perception of the presented immunology definitions expressed orally and in written form.
5. Formulate the presented immunology terms.
6. Correctly answer quiz questions.

*Attitude and Professional Behaviors.* Students should be able to:

1. Have the readiness to be hardworking.
2. Behave professionally at all times.
3. Recognize the importance of studying and demonstrate a commitment.

## 2.1 Introduction

---

The skin and mucosal level of the immune system where most immune processes take place is described. The reader can find some new data concerning the tissue-specific homeostatic cytokines, which make cells of the immune system move in the certain skin and mucosal departments. This important topic is often absent in immunology manuals.

## 2.2 Compartments of the Skin and Mucosal Immune System

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### Definitions

*Mucosae-associated lymphoid tissue (MALT)* is a collective term related to secondary lymph tissues linked to mucosae, whereas *skin-associated lymphoid tissue (SALT)* is an aggregate of T cells and other cell types in the perivascular zones of the dermis.

*Inductive site* of the MALT/SALT is a place of triggering adaptive immune responses.

*Secretory effector site* of MALT/SALT is a place where both innate and adaptive immunity's effector cells and molecules fulfill immune clearance or/and containment of pathogens.

*Secretory IgA antibodies*, dimeric molecules, are secreted by plasma cells in MALT and many secretions including breast milk. They can inhibit the colonization of pathogenic microbes on mucosae, inactivate their enzymes and toxins, and have anti-inflammatory effect.

The skin and mucosal immune system is an autonomous part of the whole immune system of the body. It consists of

1. the mucosae-associated lymphoid tissue (MALT) and
2. barrier skin and mucosal epithelium. MALT is composed of several regional compartments (see ■ Fig. 2.1):
  - (a) Tube-associated lymphoid tissue (TALT) is related to the pharynx, eustachian tube, and ear.
  - (b) Nasal-associated lymphoid tissue (NALT) belongs to the nasal cavity, mouth, oropharynx, and conjunctives.
  - (c) Bronchus-associated lymphoid tissue (BALT) is related to the trachea, bronchi, lungs, and breast glands (in females).
  - (d) Gut-associated lymphoid tissue (GALT): (i) the upper compartment controls the esophagus, stomach, and small intestine; (ii) the lower compartment controls the large intestine and proximal part of the genitourinary tract, whereas the distal part of the genitourinary tract does not have any MALT.

GALT is IL7 dependent and constituted during fetal life. Furthermore, as opposed to TALT, NALT, and BALT, GALT does not undergo age involution. Skin-associated lymphoid tissue (SALT) is related to the skin.

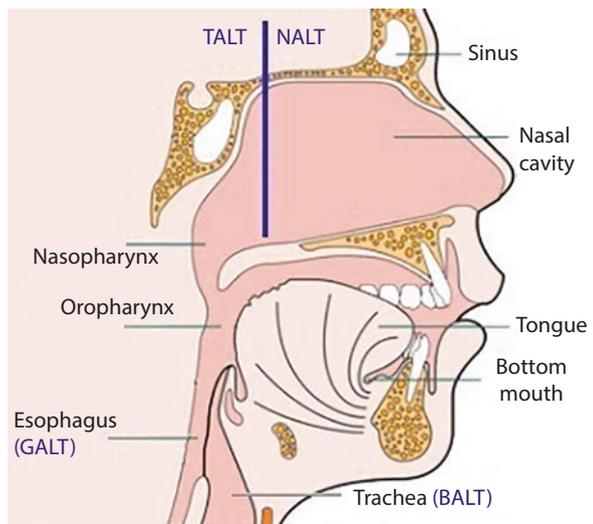
The homeostatic chemokines and their receptors, as well as cell adhesion molecules, are involved in completing the skin and mucosal sites by lymphocytes and other cells of the immune system (see ■ Table 2.1).

Cell adhesion molecules, LFA-1, ICAM-1, ICAM-2, and ICAM-3, promote lymphocyte transmigration through the vascular epithelium and homing to most tissues.

Antimicrobial factors of the skin and mucosal immune system are:

- Symbiotic (mutualistic) microbes
- Barrier epithelium with defensive qualities
- Mucus and saliva formation and keratinization

■ Fig. 2.1 Mucosae-associated lymphoid tissue (MALT)



**Table 2.1** Tissue-specific homeostatic chemokines

MALT compartments	Chemokine	Chemokine receptor	Cell adhesion molecules
Many mucosal tissues	CCL28 (MEC)	CCR10 CCR3	$\alpha_4\beta_1$ VCAM-1 $\alpha_4\beta_7$ MadCAM-1
Small intestine	CCL25 (TECK)	CCR9	$\alpha_4\beta_7$ MadCAM-1
Skin	CCL27 (CTACK)	CCR10	E-selectin P-selectin
	CCL17 (TARC)	CCR4	

- Antimicrobial substances such as lysozyme, lactic and fatty acids, etc.
- Phagocytes
- Complement
- sIgA, IgG
- Innate lymphoid cells (ILCs) including NK cells
- CD8 $\alpha$  +  $\gamma\delta$ T cells or intraepithelial lymphocytes (IELs)
- CD4 + T cells and CD8+ T cells

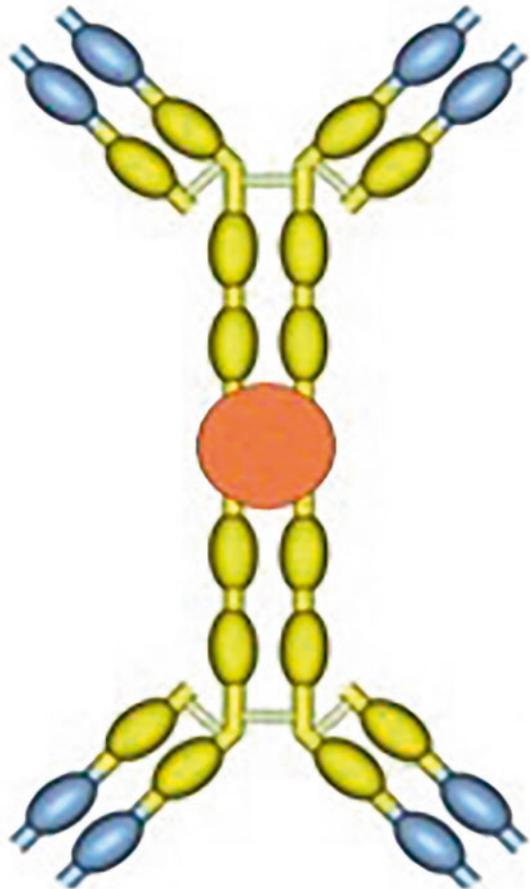
*Symbiotic microbes* such as *lactobacteria* and *bifidobacteria* play an important role in the human microbiota (or microbiome) and GALT due to their useful qualities as follows:

- Antagonism to opportunistic and absolutely pathogenic microbes
- Fermentation of dietary fibers into short-chain fatty acids such as acetic acid and butyric acid, which are both useful metabolites and bactericidal substances
- Synthesis of the enzymes, vitamins of group B, and vitamin K
- Inactivation of the toxic bile acids and xenobiotics
- Regulation of the intestinal peristalsis
- Synthesis of H<sub>2</sub>, CH<sub>4</sub>, NH<sub>3</sub>, and CO<sub>2</sub> and regulation of the interchange of gases
- Increase in the production of mucus
- Participation in the regeneration of mucosal membranes

*Secretory IgA (sIgA)* (see **Fig. 2.2**) is the most-studied classical factor of the mucosal immune system. sIgA makes up to 15% of the total number of immunoglobulins synthesized throughout the body. There are two pathways by which sIgA is synthesized, T dependent (IL10) and T independent (BAFF).

Initially, polymeric IgA (pIgA) is secreted by plasma cells and binds to polymeric immunoglobulin receptor (pIgR) on the basolateral surface of epitheliocytes becoming the dimeric IgA molecule. Epitheliocytes produce the secretory component (SC), which assists IgA in transforming through the epithelium into the lumen. SC exists in three forms: membrane SC (or pIgR), bound SC, and free SC. In addition, SC protects sIgA

■ Fig. 2.2 Secretory IgA (sIgA)



against proteolysis and acts as the glue to adhere sIgA to glycocalyx. TGF $\beta$ , IL6, IL10, and BAFF produced by epitheliocytes, T cells, and dendritic cells promote IgM antibodies switching to IgA synthesis. The elevated concentration of sIgA in mucosal secretions is a result of the cooperation between plasma cells, which secrete pIgA, becoming sIgA, and mucosal epitheliocytes, which express the pIgR.

The sIgA may exert the following effects:

- Inhibition of the colonization of pathogenic microbes
- Neutralization of the viruses and toxins
- Inhibition of the microbial enzymes
- Anti-inflammatory action

The anti-inflammatory action is associated with the low ability of sIgA to activate the complement, inactivation of phagocyte chemotaxis, inhibition of the release of TNF $\alpha$ , IL1, and IL6, and upregulation of the production of IL1ra.

**From a clinical point of view,** *selective deficiency of both serum and secretory IgA* is a primary immunodeficiency form, which occurs very often, and links to a defect in IGAD1 (IgA deficiency locus-1) on chromosome's locus 6p21.3. However, the majority of patients are clinically healthy, whereas recurrent chronic sinopulmonary, GI, and urogenital infections may occur in some persons.

### 2.2.1 Inductive and Secretory Effector Sites of MALT

The mucosal immune system functions at two types of sites: (1) inductive sites and (2) secretory effector sites. Regional MALT constitutes the *inductive sites* for mucosal immunity with their B-cell follicles and a variety of cells such as dendritic cells, macrophages, T cells, plasma cells, ILC, mast cells, etc. Naive B cells and T cells enter MALT via high endothelial venules (HEVs). MALT lacks afferent lymphatics because all exogenous antigens directly from the mucosal surfaces enter submucosa through a characteristic follicle-associated epithelium containing M cells. The M cells ("microfold cells") through which exogenous unprocessed antigens are transported to subepithelial dendritic cells that may endocytose the antigens, process, and present them along with Class I or Class II HLA molecules to lymphocytes. Antigen-specific dendritic cells may migrate via draining lymphatics to the regional lymph nodes where they can trigger adaptive immune responses. The *secretory effector sites* are located in the lumen of respiratory, GI, and genitourinary tracts. The inductive site (along with a regional lymph node) is the location for immune responses, whereas the secretory effector site is the location for effector activity of both the innate and adaptive immunities. The effector cells and molecules return here from the lymph nodes and transport from the inductive sites.

Immunoregulation at the mucosal level are mainly provided by Th9, Th17, Th22, Tfh, induced nTreg subsets, and ILC.

#### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

MALT does not include:

1. TALT.
2. NALT.
3. SALT.
4. GALT.

#### ? Question 2

This compartment of MALT is IL7 dependent:

1. GALT.
2. NALT.
3. BALT.
4. TALT.

**? Question 3**

This microbe's genus is related to symbiotic:

1. *Klebsiella* spp.
2. *Staphylococci* spp.
3. *Pseudomonas* spp.
4. *Lactobacteria* spp.

**? Question 4**

Keratinization is an antimicrobial factor of:

1. The skin.
2. The stomach.
3. The small intestine.
4. The appendix.

**? Question 5**

Phagocytosis refers to:

1. Simple B-cell-mediated response.
2. The innate immunity.
3. Apoptosis.
4. The production of mucus.

**? Question 6**

Symbiotic microbes cannot:

1. Regulate intestinal peristalsis.
2. Metabolize dietary fibers.
3. Synthesize antibodies.
4. Weaken opportunistic and absolutely pathogenic microbes.

**? Question 7**

Complement is not related to:

1. The innate immunity.
2. MALT's antimicrobial factors.
3. The adaptive immune responses.
4. The participation in inflammatory process.

**? Question 8**

Secretory IgA is:

1. Dimeric.
2. Monomeric.
3. Pentameric.
4. Septameric.

**? Question 9**

There is not a part of IgA's SC:

1. pIgR.
2. Fab.
3. Bound SC.
4. Free SC.

**? Question 10**

The pIgR is:

1. Part of immunoglobulin reagent.
2. Polymeric immunoglobulin receptor.
3. Polyspecific immunoglobulin.
4. Pentameric immunoglobulin molecule.

**? Question 11**

The sites of MALT are:

1. Antigen-binding sites.
2. Sites of inflammation.
3. Inductive and secretory effector sites.
4. Sites of chronic infections.

**? Question 12**

The inductive sites contain:

1. Cells of regional MALT.
2. Cells of the lumen.
3. The thymus.
4. The spleen.

**? Question 13**

The secretory effector sites contain:

1. Cells of regional MALT.
2. Cells of the lumen.
3. The bone marrow.
4. The spleen.

**? Question 14**

Chemokines important for the migration of cells to the skin are:

1. CCL28 (MEC).
2. CCL27 (CTACK), CCL17 (TARC).
3. CCL25 (TECK).
4. CXCL12 (SCDF-1), CCL21 (SLC).

**? Question 15**

T-independent pathway of sIgA synthesis is upregulated by:

1. BAFF.
2. IL6.
3. IL10.
4. IL1.

**? Question 16**

MALT is not present in:

1. The mouth.
2. The pharynx.
3. The small intestine.
4. The distal part of the genitourinary tract.

## 2.3 Upper and Lower Respiratory Tract and Conjunctives

### Definitions

*Tube-associated lymphoid tissue (TALT)*, a part of MALT, is the immune system of nasopharynx's mucosa, which protects the nasopharynx from various absolutely pathogenic and reactivated opportunistic microbes.

*Nasal-associated lymphoid tissue (NALT)*, a part of MALT, is the immune system of nasal mucosa, which protects the nasal cavity and mouth from airborne viruses and other absolutely pathogenic microbes and reactivated opportunistic germs.

*Pharyngeal tonsillar ring of Waldeyer-Pirogoff* is the main component of TALT and NALT, which includes unpaired *nasopharyngeal tonsil (adenoids)*, paired *tubal and palatine tonsils*, and unpaired *lingual tonsil*.

*Bronchus-associated lymphoid tissue (BALT)* is the compartment of the immune system of lower respiratory tract. BALT protects it from absolutely pathogenic microbes and, as the lower airways are not sterile, reactivated opportunistic germs.

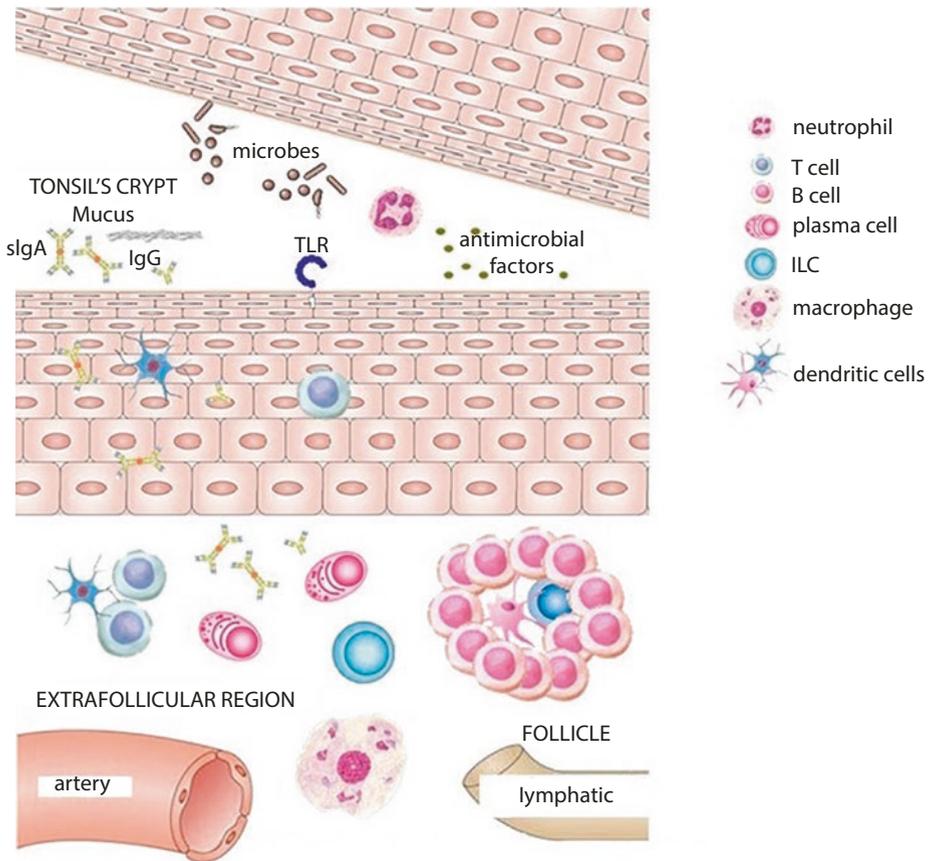
*Tonsils* are lymphoid aggregates in the upper respiratory organs and mouth (see ■ Table 2.2). They are completely encapsulated, covered by two types of epithelium, have M cells for antigen capture on their surface, and lack afferent lymphatics.

The tonsils capture antigens through M cells in their crypt epithelium or non-crypt epithelial surface, whereas the majority of lymph nodes receive antigens via the bloodstream and lymph flow. However, the crypts mainly belong to the secretory effector sites, where there are microbes, mucus, sIgA, IgG, lysozyme, complement proteins, neutrophils, etc. (see ■ Fig. 2.3). The tonsil's epithelium contains HEV and, as a consequence, some cells of the immune system including intraepithelial lymphocytes (IELs) and Langerhans cells. IELs are represented by CD8 $\alpha\alpha$  + T cells, i.e., T cells, which express  $\gamma\delta$ TCR instead of  $\alpha\beta$ TCR. Epitheliocytes display TLR and other PRRs.

The tonsil's follicles are called lymphoid nodules. They are B-cell areas where there are also follicular dendritic cells (fDCs) and follicular helper T cells (Tfh) to provide the advanced B-cell-mediated immune responses. Constituted plasma cells produce sIgA and IgG (sIgA > IgG). The antibodies through the epithelium are released into the crypts

**Table 2.2** Pharyngeal tonsillar ring of Waldeyer-Pirogoff

Tonsil	MALT's compartment	Epithelium type	Crypts
Unpaired nasopharyngeal tonsil (adenoids)	TALT	Ciliated columnar epithelium	Absent
Paired tubal tonsils	TALT	Ciliated columnar epithelium	Absent
Paired palatine tonsils	NALT	Nonkeratinized stratified squamous epithelium	Present
Unpaired lingual tonsil	NALT	Nonkeratinized stratified squamous epithelium	Present



**Fig. 2.3** Structure of the palatine tonsil

or the space near the tonsil's surface. The tonsils are characterized by the high number of B cells and secreted immunoglobulins as compared to any other lymphoid organ of the immune system ( $B > T$ ).

Subepithelial dendritic cells are represented by type 1 (myeloid, mDC) and type 2 (plasmacytoid, pDC) dendritic cells. All subsets of these cells are immature when involved in endocytosis and processing antigens. During maturation, the dendritic cells acquire characteristic long outgrowths called dendrites, which are necessary for antigen presentation. There are also macrophages, neutrophils, and ILC.

Small extrafollicular regions (T-cell areas) contain CD4 + T cells, CD8 + T cells, interdigitating dendritic cells, and macrophages. Here is the location for the T-cell-mediated immune responses.

TALT's/NALT's cells migrate to the draining cervical and submandibular lymph nodes.

**From a clinical viewpoint,** tonsils may be enlarged (*adenotonsillar hyperplasia*), inflamed (*tonsillitis* and *adenoiditis*), and complicated (*peritonsillar abscess*) and, in some cases, may require surgical intervention (*drain the pus in the abscess, adenotomy, and tonsillectomy*).

The BALT structure is similar to the TALT structure. The lower airways are covered by the ciliated columnar epithelium, which also contains M cells, goblet cells, CD8 $\alpha\alpha$  + IEL, and Langerhans cells. The goblet cells produce the mucus. Subepithelial dendritic cells are represented by type 1 and type 2 dendritic cells. Interestingly, here are many mast cells and eosinophils. Follicles (B-cell areas) comprise B cells, fDCs, Tfh, and plasma cells. Extrafollicular regions (T-cell areas) contain CD4 + T cells, CD8 + T cells, interdigitating dendritic cells, and macrophages.

BALT's cells migrate to the draining mediastinal lymph nodes.

### ■ Quiz

Reading a question, please choose only one right answer.

#### ❓ Question 1

Do tonsils have afferent lymphatics?

1. Yes.
2. No.
3. Lingual tonsil only.
4. Adenoids only.

#### ❓ Question 2

Predominant cell types in NALT/TALT are:

1. B cells.
2. CD4 + T cells.
3. Macrophages.
4. CD8 + T cells.

**? Question 3**

The palatine tonsil must be:

1. Unpaired.
2. Covered by ciliated columnar epithelium.
3. Covered by keratinized stratified squamous epithelium.
4. Paired.

**? Question 4**

The tubal tonsils must be:

1. Unpaired.
2. Covered by ciliated columnar epithelium.
3. Covered by non-keratinized stratified squamous epithelium.
4. Covered by keratinized stratified squamous epithelium.

**? Question 5**

The tonsils capture antigens through:

1. CD4 + T cells.
2. M cells.
3. CD8 + T cells.
4. Plasma cells.

**? Question 6**

The tonsil's follicles are:

1. CD8 + T-cell zones.
2. CD4 + T-cell zones.
3. B-cell zones.
4. Macrophage zones.

**? Question 7**

Predominant immunoglobulin class in NALT/TALT/BALT is:

1. IgM.
2. IgG.
3. Secretory IgA.
4. IgA.

**? Question 8**

Follicular helper T (T<sub>fh</sub>) cells are located in:

1. The tonsil's follicles.
2. The barrier epithelium.
3. The tonsil's extrafollicular region.
4. The tonsil's crypt.

**? Question 9**

Antimicrobial factors are present in:

1. The tonsil's extrafollicular region.
2. The tonsil's crypts.
3. The tonsil's follicles.
4. The cervical lymph nodes.

**? Question 10**

The tonsils are characterized by:

1. IL7 dependency.
2. Age involution.
3. Non-encapsulated structure.
4. Presence of afferent lymphatic vessels.

**? Question 11**

The BALT's structure is similar to:

1. GALT's structure.
2. NALT's structure.
3. TALT's structure.
4. SALT's structure.

**? Question 12**

Intraepithelial lymphocytes are:

1. CD8 +  $\alpha\alpha$   $\gamma\delta$ T cells.
2. Langerhans cells.
3. T cells with  $\alpha\beta$ TCR.
4. B cells.

**? Question 13**

The tonsil's epithelium express:

1.  $\alpha\beta$ TCR.
2. BCR.
3. TLRs and other PRRs.
4.  $\gamma\delta$ TCR.

**? Question 14**

The tonsil's crypts or the space near the crypt-absent tonsil's surface are related to:

1. MALT's inductive sites.
2. MALT's secretory effector site.
3. Lymph flow.
4. Blood stream.

**? Question 15**

The tonsil's follicles and extrafollicular regions are related to:

1. MALT's inductive sites.
2. MALT's secretory effector site.
3. Blood stream.
4. Lymph flow.

**? Question 16**

As a rule, BALT's cells migrate to:

1. The spleen.
2. Draining cervical lymph nodes.
3. Draining submandibular lymph nodes.
4. Draining mediastinal lymph nodes.

## 2.4 Mouth and Gastrointestinal and Genitourinary Tracts

### 2

#### Definitions

*GALT-associated lymphoid tissue (TALT)*, a IL7-dependent part of MALT, is the immune system of gastrointestinal mucosa, which protects the gastrointestinal tract from various absolutely pathogenic and reactivated opportunistic microbes.

*Peyer's patches* related to GALT are lymphoid aggregates in the small intestine. They contain a variety of cell types including B-cell follicles.

*Solitary (isolated) follicles* related to GALT are lymphoid aggregates in both the small and large intestines. Their structure is similar to Peyer's patches.

*Appendix* related to GALT is a secondary lymphoid organ, which is important for the digestive defense and homeostasis. Nowadays, the appendix must not be considered longer as a vestigial organ.

### 2.4.1 Mouth

Mouth immunity is supported by NALT, barrier epithelium, dental defensive factors, and salivary glands.

A nonkeratinized stratified squamous epithelium covers the soft palate, inner lips, cheeks, bottom mouth, and ventral surface of the tongue. *Mucus formation* is characteristic for the nonkeratinized epitheliocytes and directed against the colonization of reactivated opportunistic microbes, including cariogenic bacteria such as *Streptococcus mutans*, *Lactobacillus acidophilus*, *Actinomyces viscosus*, etc. and absolutely pathogenic germs.

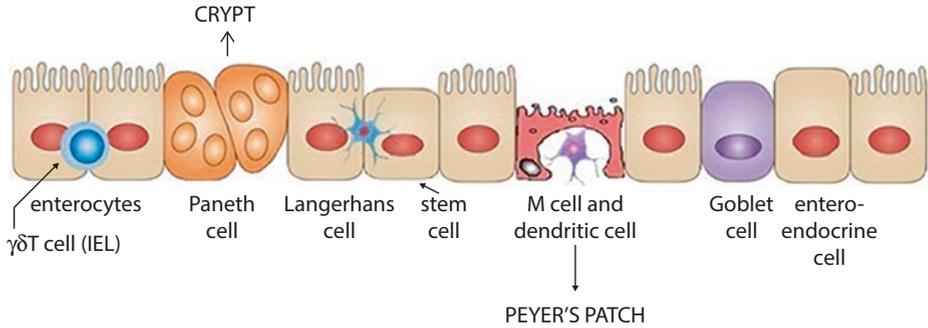
A keratinized stratified squamous epithelium covers the gums, hard palate, and dorsal surface of the tongue. This type of epithelium possesses some layers such as basal, spinous, granular, and cornified. *Keratinization* related to the keratinized stratified squamous epithelium is also an essential factor of defense against microbial colonization.

Teeth have *self-dental immune defense* including the enamel, periodontal ligament, and gingival crevicular fluid. The periodontal ligament acts as a protective membrane, which defends the periodontium against activated opportunistic microbes such as *Porphyromonas gingivalis* and other parodontogenic pathogens. The gingival crevicular fluid is not a product of the mucosal immunity but is derived from the blood. It is accumulated by transudation from the gingival capillary network and epithelium around the dental neck. The gingival crevicular fluid is rich in antimicrobial factors of the blood.

*Saliva formation* is a result of the function of the salivary glands. The saliva creates a beneficial microenvironment in the mouth because it contains a variety of humoral and cellular components of the mucosal immune system, in particular, sIgA, IgG, IgM, lactoferrin, lysozyme, agglutinins, lactoperoxidase, complement proteins, neutrophils, etc.

### 2.4.2 Gastrointestinal Tract

GALT includes small scattered lymphoid elements in the esophagus and stomach, Peyer's patches in the small intestine, appendix, and solitary (isolated) follicles in both the small and large intestine.



■ Fig. 2.4 Small intestine epithelium

The GI tract is covered by simple columnar epithelium with microvilli and associated with a layer of glycocalyx on their luminal surface to protect epitheliocytes from the acid pH. Among epitheliocytes, there are many cell types, but enterocytes and colonocytes are the dominant types (see ■ Fig. 2.4). The epithelium sits on the underlying connective tissue called lamina propria, constituting villi and crypts, while neutrophil-like Paneth cells are located on the crypt's bottom. The Paneth cells release many antibacterial factors providing the crypts with a sterile condition. Almost every week, a new epitheliocyte regenerates from stem cells inbuilt in the epithelial monolayer. The other cell types are goblet cells, M cells, CD8 $\alpha$  +  $\gamma$  $\delta$ T cells (IELs), Langerhans cells, and enteroendocrine cells. The goblet cells secrete 30  $\mu$ m one-layer mucus in the small intestine and 480  $\mu$ m two-layer mucus in the large intestine, and the mucus may serve as a “trap” for microbes to inhibit their colonization.

*Peyer's patches, solitary (isolated) follicles, and the appendix* are aggregated lymphoid follicles of the GI tract. The scattered lymphoid elements in the esophagus and stomach do not organize similar aggregates. In the aggregated lymphoid follicles, there are B-cell areas where fDCs and Tfh are also present to take part in the advanced B-cell-mediated immune response. Formed plasma cells secrete IgG and IgA, which are transported into the lumen by means of SC (*sIgA* > *IgG*). Some antigen-specific dendritic cells may migrate via draining lymphatics to mesenteric lymph nodes where they can also trigger an advanced B-cell-mediated immune response.

The structure of the lamina propria is very compressible and elastic, which allows it to support the nourishment of epithelium and contains a variety of cell types. There are cells of lymphoid aggregates such as Peyer's patches, type 1 dendritic cells (myeloid, mDC) and type 2 dendritic cells (plasmacytoid, pDC), Langerhans cells, fibroblasts, macrophages, neutrophils, mast cells, and eosinophils.

Analogous to TALT/NALT, small extrafollicular regions (T-cell areas) contain CD4 + T cells, CD8 + T cells, interdigitating dendritic cells, and macrophages. Here is the location for T-cell-mediated immune responses.

The lumen is the secretory effector site of GALT. The most significant number of microbiota's microbes is located in the GI tract, which creates a load of responsibility for the GI tract's mucosal immunity.

**From a clinical viewpoint,** defects in the mucosal immune system may lead to *Helicobacter pylori*-associated gastritis and peptic ulcer disease, the intestinal bacterial overgrowth syndrome, traveler's diarrhea, pseudomembranous colitis, and irritable

*bowel syndrome*. *Appendicitis* is a disease characterized by inflammation of the appendix, which requires urgent surgical removal to prevent such inflammation to progress as peritonitis or even sepsis.

### 2.4.3 Genitourinary Tract

The mucosal immune system of the genitourinary tract has some peculiarities, as follows:

- The absence of organized lymphoid follicles in the distal part of the genitourinary tract
- The predominance of IgG over sIgA in lumen's secretions ( $IgG > sIgA$ )
- Regulatory influence of sex hormones

The proximal part is supported by GALT.

Normal vaginal microbiota includes Döderlein's flora, which consists of *Lactobacteria*, *Bifidobacteria*, and *Peptostreptococci*. Döderlein's flora makes up to 80–95% of the whole vaginal microbiota and plays an essential role in the maintenance of vaginal homeostasis. *Lactobacteria* are the most important mutualistic microbes producing lactic acid and hydrogen peroxide that decrease vaginal pH and protect the female genitourinary tract against the colonization of pathogenic species. In healthy females, *Lactobacteria* must make up  $10^8$ – $10^{12}$  CFU/mL or not less than 80% of the vaginal microbial mass. If this parameter is lower, bacterial vaginosis occurs. The lumens of the vagina, cervix, and urethra represent the secretory effector sites of the mucosal immune system.

A nonkeratinized stratified squamous epithelium covers distal parts of the genitourinary tract in women and men. Langerhans cells and CD8 $\alpha\alpha$  +  $\gamma\delta$ T cells are inbuilt in the epithelium, which expresses TLR. This type of epithelium enables to produce mucus as a factor of the innate immunity. A keratinized stratified squamous epithelium covers the glans penis in circumcised men, which exhibits another factor of the innate immunity, keratinization. Uncircumcised males lack this protective factor. If keratinization is taken into consideration, the foreskin is "Achilles' heel" of male innate immunity.

Under the epithelial cover, there are many cells of the immune system including macrophages, NK cells, dendritic cells, CD4 + T cells, CD8 + T cells, etc., which take part in immune processes. It is the inductive site of the genitourinary tract. The cells function here and may migrate to the draining inguinal lymph nodes.

**From a clinical viewpoint,** any imbalances in the mucosal immune system, in both females and males, promote a variety of diseases of the genitourinary tract. Bacterial vaginosis in women increases the transmission risk of some sexually transmitted infections, including HIV, and the risk of preterm birth in pregnant women. More serious problems are chronic recurrent colpitis, cervicitis, and pelvic inflammatory disease including abscesses. In males, there may occur an elevated transmission risk of sexually transmitted infections to sexual partners, chronic recurrent urethritis, prostatitis, epididymitis, and oligozoospermia.

**■ Quiz**

Reading a question, please choose only one right answer.

**? Question 1**

Development of GALT is characterized by:

1. IL7 dependency.
2. Age involution.
3. Non-encapsulated structure only.
4. Absence of goblet cells.

**? Question 2**

Solitary (isolated) follicles are present in:

1. The small and large intestines.
2. The small intestine only.
3. The large intestine only.
4. The stomach.

**? Question 3**

Goblet cells produce:

1. Hormones.
2. Antibodies.
3. Lysozyme.
4. Mucus.

**? Question 4**

Paneth's cells secrete:

1. Antimicrobial factors.
2. Mucus.
3. Saliva.
4. Lymph.

**? Question 5**

GALT captures antigens through:

1. CD4 + T cells.
2. Goblet cells.
3. CD8 + T cells.
4. M cells.

**? Question 6**

The place of T-cell-mediated responses in GALT is:

1. Peyer's patches.
2. Intestine's lumen.
3. Extrafollicular regions.
4. GI tract's simple columnar epithelium.

**? Question 7**

The place of B-cell-mediated responses in GALT is:

1. Peyer's patches.
2. Intestine's lumen.
3. Extrafollicular regions.
4. GI tract's simple columnar epithelium.

**? Question 8**

Lamina propria is located in:

1. The space under the basement membrane of intestinal epithelium.
2. The intestinal lumen.
3. The intestinal crypts.
4. The intestinal simple columnar epithelium.

**? Question 9**

Intraepithelial lymphocytes are:

1. CD8 +  $\alpha\alpha$   $\gamma\delta$ T cells.
2. Langerhans cells.
3. T cells with  $\alpha\beta$ TCR.
4. Type 2 dendritic cells.

**? Question 10**

Predominant immunoglobulin class in the genitourinary tract is:

1. IgM.
2. IgG.
3. Secretory IgA.
4. IgA.

**? Question 11**

The mucosal immune system of the genitourinary tract is characterized by:

1. Presence of organized follicles in the distal part.
2. Absence of any organized follicles.
3. Absence of organized follicles in the distal part.
4. Absence of organized follicles in the proximal part.

**? Question 12**

Uncircumcized males:

1. Have less defense against sexually transmitted viruses.
2. Have more defense against sexually transmitted viruses.
3. Have the same defense as compared to circumcized males.
4. Have the best defense against transmission of *HIV*.

**? Question 13**

Bacterial vaginosis is caused by:

1. *Neisseria gonorrhoeae*.
2. *Chlamydia trachomatis*.
3. Imbalances in the mucosal immune system of the genitourinary tract in females and reactivation of opportunistic microbes.
4. *Treponema pallidum*.

**? Question 14**

As a rule, cells from the mucosal immune system of the genitourinary tract migrate to:

1. The draining mediastinal lymph nodes.
2. The draining inguinal lymph nodes.
3. The spleen.
4. The draining submandibular lymph nodes.

**? Question 15**

According to problems linked to *HIV* and *HPV*, the circumcision in males may be considered as a useful measure:

1. Yes.
2. No.
3. Never.
4. Unknown.

**? Question 16**

The secretory effector sites of the genitourinary mucosal immune system are:

1. Genitourinary nonkeratinized stratified squamous epithelium.
2. Genitourinary keratinized stratified squamous epithelium.
3. The inguinal lymph nodes.
4. The lumens of the vagina, cervix, and urethra.

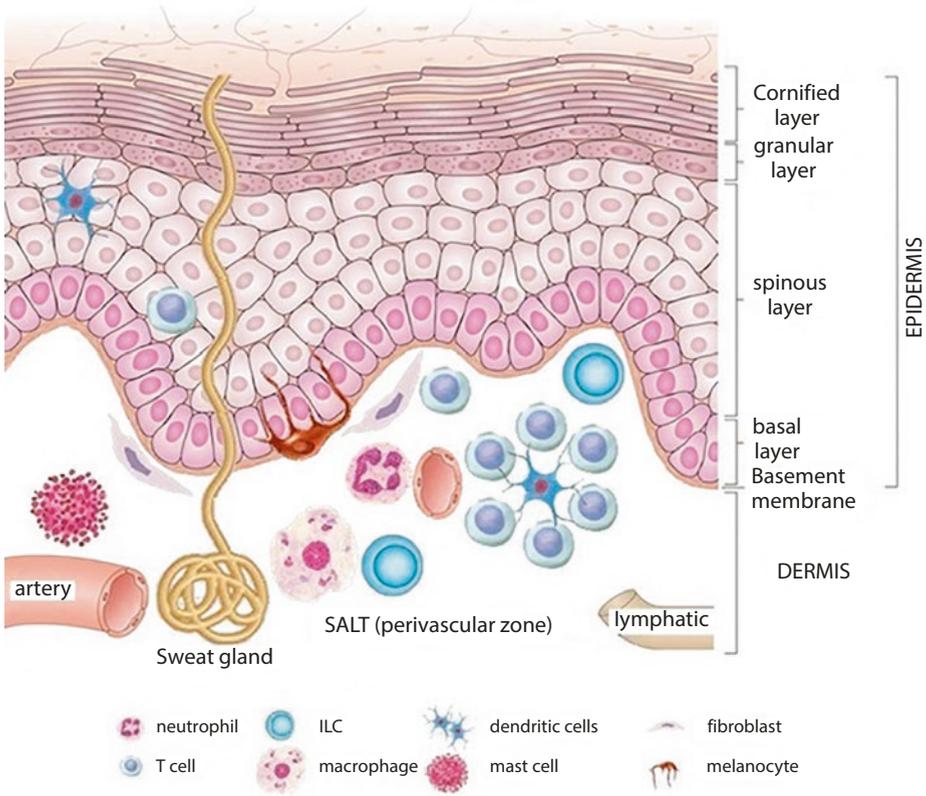
## 2.5 Skin

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**Definitions**

*Skin* is a secondary organ of the immune system covering the human body and containing lots of T cells as well as other cell types and defensive molecules.

*Keratinization* is a protective factor of the skin associated to filaggrin, a protein of keratinocytes.



■ Fig. 2.5 Structure of the skin

The *skin* is the largest organ in the body. In adults, the skin has a surface area of between 1.5–2.0 m<sup>2</sup>. Total skin weight makes up about 15% of the body's weight. The skin is a secondary organ of the immune system (see ■ Fig. 2.5). The autonomous skin immune system consists of (1) the epidermis with immunologically active keratinocytes, Langerhans cells, and CD8 $\alpha\alpha$  +  $\gamma\delta$ T cells (IELs) and (2) skin-associated lymphoid tissue (SALT) located in perivascular zones of the dermis. Interestingly, the skin is full of memory T cells.

The epidermis is represented by a keratinized stratified squamous epithelium, which consists of mitotically active cells, keratinocytes, capable of keratinization. The epidermis located on the basement membrane is composed of some layers, basal, spinous, granular, and cornified.

*Keratinization* is the process of the differentiation of keratinocytes, epitheliocytes of the epidermis. In the basal layer, keratinocytes proliferate by means of mitosis, and the new cells move up the next layers, changing shape and composition and losing the nucleus. Gradually, keratinocytes form desmosomes, cellular junctions, which are important for skin mechanical strength, and release keratin proteins and lipids with defensive qualities. One of the proteins, filaggrin, is a filament-associated barrier protein of keratinocytes, vital for keratinization. Eventually, keratinocytes from the cornified layer are shed from the surface and removed along with microbes. Besides,

keratinocytes may secrete IL1, IL6, IL8, IFN $\alpha$ , IFN $\beta$ , TNF $\alpha$ , G-CSF, and GM-CSF and function as antigen-presenting cells capable of processing antigens and presenting them in association with Class II HLA molecules.

**From a clinical viewpoint,** some mutations are described in the filaggrin gene on 1q21.3 that result in *psoriasis*, *atopic dermatitis*, *ichthyosis vulgaris*, etc. So, the keratinocytes and keratinization are essential factors of the innate and adaptive immunity.

The dermis consists of two layers: the papillary region and reticular region. The papillary region is composed of finger-like papillae, which extend to the epidermis. The reticular region located deeper contains the dense concentration of elastic, collagenous, and reticular fibers. Here are the sweat glands, sebaceous glands, and roots of the hair and nails. The blood vessels are available in both regions of the dermis, forming the perivascular zones for SALT.

SALT comprises 90% of the total number of skin T cells, of which 99% are  $\alpha\beta$ TCR-bearing T cells and only 1% are  $\gamma\delta$ T cells. Among T cells, there are memory CD45R0+ T ( $T_{EM}$ ) lymphocytes and type 1, type 2, type 17, and type 22 helper CD4+ T cells. The interdigitating dendritic cells are located in the center of these lymphoid aggregates, whereas ILC including NK cells, dermal dendritic cells, macrophages, eosinophils, mast cells, fibroblasts, and melanocytes are disposed outside the SALT. Interestingly, a minor part of skin lymphocytes is composed by B cells, but they may enter the skin through blood and lymphatic vessels ( $T > B$ ).

Cutaneous lymphocyte-associated antigen (CLA antigen) is expressed by a subset of effector memory T ( $T_{EM}$ ) cells, which are recruited in the pathogenesis of different T-cell-mediated diseases of the skin such as atopic and contact dermatitis and psoriasis. CLA + T cells are also resident in normal skin, under resting conditions.

Mast cells are mainly represented by a connective tissue phenotype, involved in the early phase of type I hypersensitivity, along with eosinophils and neutrophils, which are necessary for the late phase.

Macrophages play many roles in the skin immune system. They phagocyte microbes and damaged cells and secrete a number of biologically active substances including cytokines and chemokines and reactive oxygen species (ROS) including nitric oxide, matrix metalloproteinases, and arachidonates. Macrophages may upregulate type 1 helper T cells' polarization condition, activate fibroblasts and keratinocytes, and take part in type IV hypersensitivity and CD4 + T-cell-mediated immune response.

All cells may leave the skin and migrate to the draining regional lymph nodes.

## ■ Quiz

Reading a question, please choose only one right answer.

### ? Question 1

Predominant cells in the skin except keratinocytes are:

1. T cells.
2. B cells.
3. Langerhans cells.
4. Neutrophils.

**? Question 2**

The skin is covered by:

1. Simple ciliated columnar epithelium.
2. Keratinized stratified squamous epithelium.
3. Nonkeratinized stratified squamous epithelium.
4. Pseudostratified columnar epithelium.

**? Question 3**

In uncircumcized males, glans penis is covered by:

1. Keratinized stratified squamous epithelium.
2. Simple cuboidal epithelium.
3. Simple ciliated columnar epithelium.
4. Nonkeratinized stratified squamous epithelium.

**? Question 4**

The keratinization is related to:

1. Defensive factors of the skin.
2. Simple B-cell-mediated response.
3. Phagocytosis.
4. Complement activation.

**? Question 5**

SALT does not include:

1. Helper T cells.
2. Interdigitating dendritic cells.
3. Macrophages.
4. Memory T cells.

**? Question 6**

CLA + T cells may be involved in the pathogenesis of:

1. Furunculosis.
2. Acne.
3. Atopic dermatitis and psoriasis.
4. Hydradenitis and skin abscess.

**? Question 7**

Langerhans cells are present in/on:

1. Dermis.
2. Skin surface.
3. Epidermis.
4. Macrophages.

**? Question 8**

Langerhans cells refer to:

1. Dendritic cells.
2. B cells.
3. Macrophages.
4. Memory T cells.

**? Question 9**

The epidermis does not comprise:

1. Keratinocytes.
2. B cells.
3. Langerhans cells.
4. CD8 +  $\alpha\alpha$   $\gamma\delta$ T cells.

**? Question 10**

The skin is:

1. A primary immune organ.
2. A secondary immune organ.
3. An organ responsible for fertility.
4. A tertiary immune organ.

**? Question 11**

Keratinocytes may be considered as:

1. Memory CD8 + T cells.
2. Memory CD4 + T cells.
3. Antigen-presenting cells.
4. Memory B cells.

**? Question 12**

SALT is located in:

1. Perivascular zones of the dermis.
2. Spinous layer of the epidermis.
3. Basal layer of the epidermis.
4. The sweat glands.

**? Question 13**

Mast cells are present in the skin:

1. No.
2. Unknown.
3. Yes.
4. No way.

**? Question 14**

Filaggrin is:

1. A protein of melanocytes.
2. A barrier protein of keratinocytes.
3. A defensive factor of neutrophils.
4. A defensive factor of macrophages.

**? Question 15**

Filaggrin's mutations may result in a skin pathology:

1. No way.
2. Unknown.
3. Yes.
4. Never.

**? Question 16**

Reactive oxygen species (ROS) may be produced in:

1. CD8 + T cells and CD4 + T cells.
2. B cells.
3. NK cells.
4. Macrophages and neutrophils.

**Key points**

1. The immune system of the barrier organs, or immune system at the local level, plays a crucial role in the defense during the human everyday life since exogenous invaders and reactivated opportunistic pathogens first attack the skin and mucosae and can cause damage to barrier tissues.
2. The skin and mucosal immune system includes all types of covering and lining epithelium, and SALT/MALT, containing  $\gamma\delta$ T cells, some types of dendritic cells, phagocytes, mast cells, eosinophils, and other cell lineages. The cells secrete a variety of molecules including antimicrobial peptides, cytokines, chemokines, immunoglobulins, etc. Immunoregulation at the local level is mainly provided by Th9, Th17, Th22, Tfh, induced nTreg, and ILC.
3. MALT is divided into some compartments, TALT, NALT, BALM, and GALT, which are well-structured, in particular GALT, and two different functional sites, inductive site and secretory effector site.

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# Innate Immunity

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**Electronic supplementary material** The online version of this chapter ([https://doi.org/10.1007/978-3-030-03323-1\\_3](https://doi.org/10.1007/978-3-030-03323-1_3)) contains supplementary material, which is available to authorized users.

## Learning Objectives

*Knowledge.* Upon successful completion of the chapter, students should be able to:

1. Distinguish between innate and adaptive immunity.
2. List the main mechanisms of innate immunity.
3. Explain the postulates of pattern recognition theory.
4. Describe the “acute phase” proteins and their action.
5. Describe the complement system’s structure, activation pathways, and function.
6. Define the phagocytosis and NETosis.
7. Explain how the interferons and NK cells protect against invaders and tumor cells.
8. Be familiar with the inflammasomes, pyroptosis and types of inflammatory processes.

*Acquired Skills.* Upon successful completion of the chapter, students should demonstrate the following skills, including:

1. Interpret the knowledge related to innate immunity.
2. Critically evaluate the scientific literature about the mechanisms of innate immunity.
3. Discuss the scientific articles from the current research literature to criticize experimental data and formulation of new hypotheses in basic immunology.
4. Attain a clear perception of the presented immunology definitions expressed orally and in written form.
5. Formulate the presented immunology terms.
6. Correctly answer quiz questions.

*Attitude and Professional Behaviors.* Students should be able to:

1. Have the readiness to be hardworking.
2. Behave professionally at all times.
3. Recognize the importance of studying and demonstrate a commitment.

## 3.1 Introduction

---

The first line of defense is the innate immunity including phagocytosis, NETosis, natural cytotoxicity, pyroptosis, the formation of inflammasomes, etc. The reader can find the explanation of the pattern recognition theory, a novel concept in immunology. Clinical comments include the description of primary immunodeficiencies on the base of mutations in genes important for the innate immunity and the features of acute physiological, acute pathological, and chronic pathological inflammation.

Innate immunity is the first line of defense, which is activated very soon after pathogen exposure or opportunistic microbe reactivation. B.A. Beutler, J.A. Hoffmann, and R.M. Steinman were awarded the Nobel Prize in 2011 for research in the field of innate immunity including TLR and discovering key principles in the activation of the immune system.

### 3.3 • “Acute Phase” Proteins

■ **Fig. 3.1** C.A. Janeway Jr. (1943–2003)



## 3.2 Pattern Recognition Theory

---

C.A. Janeway, Jr. (see ■ Fig. 3.1), and his colleagues revised the working model of the immune system from the age of Burnet’s clonal selection theory. It is now clear that antigen-presenting cells recognize conserved microbial components, pathogen-associated molecular patterns (PAMPs) such as bacterial lipopolysaccharides and peptidoglycans, as well as viral ssRNA and dsRNA. These PAMPs serve as ligands for a broad array of protein families referred to as pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs), RIG-1-like receptors (RLRs), and AIM-2-like receptors (ALRs). When a PRR on an antigen-presenting cell binds to an appropriate PAMP, this cell begins to present antigen (signal 1), stimulate the expression of costimulatory molecules (signal 2), and secrete cytokines (signal 3), which is required for the course of an adaptive immune response to the causative pathogen. Thus, known PRRs are explicitly being triggered by adding or conjugating PAMPs to antigens of interest.

Since the germline-encoded PRRs are selected during evolution to detect microbial PAMPs, which are not produced by multicellular hosts, they can efficiently discriminate “self” from microbial “non-self.” Their recognition by PRRs means the presence of microbial “non-self,” commonly a pathogen, and triggers both the urgent innate immunity and the adaptive immune responses.

Nowadays, modern immunology is based on two major paradigms: clonal selection theory and pattern recognition theory. Both paradigms were developed initially on theoretical grounds and experimentally proven years later.

## 3.3 “Acute Phase” Proteins

---

### Definitions

*Acute phase proteins* are the bloodstream proteins, which are associated to the onset of any infection or tissue injury. They may be either increased (*positive*) or decreased (*negative*) and either *pro-inflammatory* or *anti-inflammatory*.

After pathogens invade the body or in a case of tissue injury, some special proteins, “acute phase” proteins, rapidly flood the bloodstream. As a rule, they are defined as proteins that change their serum concentration by >25% in response to pro-inflammatory cytokines, IL1 $\beta$ , IL6, and TNF $\alpha$ . Such increased proteins are called *positive*. The liver produces the majority of the positive “acute phase” proteins. At the same time, the production of the number of other proteins is decreased, and they are termed *negative*. On the other hand, some “acute phase” proteins enhance inflammation very much (*pro-inflammatory* “acute phase” proteins) and may even contribute to the promotion of sepsis, and other proteins are always *anti-inflammatory*.

### 3.3.1 Pro-inflammatory “Acute Phase” Proteins

---

*C-reactive protein (CRP)* is a pentameric protein capable of binding to lysophosphatidylcholine expressed on the surface of bacteria, an activating complement via a C1q subcomponent, and enhancing phagocytosis through opsonization. An increase in CRP occurs within 2 h of the onset of inflammation, up to 50,000-fold, and peaks at 48 h.

*Mannose-binding lectin (MBL)*, an oligomeric protein, belongs to the collectin subgroup of the C-type lectin superfamily. It binds carbohydrate “patterns” on the surface of bacteria, fungi, viruses, and protozoans; activates complement via the lectin subpathway, a variant of the classical pathway; and may take part in opsonization.

*Surfactant protein A (SP-A)* and *surfactant protein D (SP-D)* refer to the collectin subgroup of the C-type lectin superfamily, which can recognize “patterns.” SP-A and SP-D are capable of binding bacterial lipopolysaccharides (LPSs) and fungal glucan and mannose residues, enhancing phagocytosis by means of opsonization, and improving the clearance of lung pathogens.

*L-, H-, and M-ficolins*, novel proteins, are related to the collectin subgroup of the C-type lectin superfamily. It is known that they can bind a wide range of carbohydrate “patterns” on the microbial surfaces and take part in complement activation via the lectin subpathway.

Cytokines *IL1 $\beta$* , *IL6*, and *TNF $\alpha$*  and fragments of activated complement such as *C2a*, *C3a*, *C4a*, and *C5a* may also be related to the positive pro-inflammatory “acute phase” proteins.

### 3.3.2 Anti-inflammatory “Acute Phase” Proteins

---

*Serum amyloid A (SAA)*, an apolipoprotein, is a monomer “acute phase” protein but may turn into a polymer under such a pathological condition as amyloidosis. In acute inflammation, SAA plays the role of an “urgent bandage” on injured tissue. SAA arises within hours after an inflammatory stimulus, and the magnitude of its increase may be enormous.

$\alpha_2$ -*Macroglobulin*, the large serum protein, acts as an antiprotease, downregulator of fibrinolysis, and inhibitor of thrombin. In acute inflammation, the concentration of  $\alpha_2$ -macroglobulin may enhance fivefold and more.

$\alpha_1$ -Antitrypsin can inhibit a wide variety of proteases and protect tissues from injury. In acute inflammation, its concentration can be elevated manyfold. Deficiency of  $\alpha_1$ -antitrypsin may be fatal and lead to the loss of lung elasticity and progression of severe emphysema.

*Ceruloplasmin* is a ferroxidase enzyme that oxidizes iron. Respectively, it inhibits iron uptake by microbes. Furthermore, it carries more than 95% of the total copper in healthy human serum.

*Fibrinogen*, a component of the coagulation system, is converted by thrombin into fibrin during blood clot formation. In acute inflammation, the coagulation system protects tissue from bleeding. Fibrinogen may be elevated 1.5--2-fold when any form of acute inflammation occurs.

*Haptoglobin* binds free hemoglobin released from erythrocytes, which may take place during acute inflammation and thereby prevent loss of iron through the kidneys.

**From a clinical viewpoint,** the positive “acute phase” proteins are used as markers of any inflammation in the body. Some of them ( $\alpha_2$  macroglobulin, fibrinogen) are useful for evaluating blood coagulation, intravascular hemolysis, and thrombosis. Deficiency of  $\alpha_1$ -antitrypsin is a diagnostic marker of *congenital emphysema*.

#### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

The most increased “acute phase” protein is:

1. CRP.
2. Fibrinogen.
3. MBL.
4. SAA.

#### ? Question 2

This “acute phase” protein is not pro-inflammatory:

1. SAA.
2. CRP.
3. SP-A.
4. MBL.

#### ? Question 3

This “acute phase” protein is not anti-inflammatory:

1. Fibrinogen.
2. SAA.
3. Haptoglobin.
4. CRP.

**? Question 4**

Deficiency of  $\alpha_1$ -antitrypsin results in:

1. Congenital emphysema.
2. Bronchial asthma.
3. Atopic dermatitis.
4. Ichthyosis vulgaris.

**? Question 5**

This “acute phase” protein is not related to the C-type lectin superfamily:

1. SP-D.
2. CRP.
3. MBL.
4. L-ficolin.

**? Question 6**

This “acute phase” protein is related to the coagulation system:

1. Ceruloplasmin.
2. SAA.
3. Fibrinogen.
4. CRP.

**? Question 7**

This cytokine may be considered as “acute phase” protein:

1. IL4.
2. IL10.
3. IL6.
4. IFN $\alpha$ .

**? Question 8**

This cytokine may not be considered as “acute phase” protein:

1. IL10.
2. IL6.
3. IL1 $\beta$ .
4. TNF $\alpha$ .

**? Question 9**

The complement system can produce “acute phase” proteins:

1. No way.
2. Yes.
3. No.
4. Sometimes.

**? Question 10**

The term “positive proteins” means:

1. They are decreased in inflammation.
2. They are elevated in inflammation.

## 3.3 • “Acute Phase” Proteins

3. They are not changed in inflammation.
4. Unknown.

**? Question 11**

The term “negative proteins” stands for:

1. Unknown.
2. They are elevated in inflammation.
3. They are not changed in inflammation.
4. They are decreased in inflammation.

**? Question 12**

In inflammation and tissue injury, “acute phase” proteins flood the bloodstream:

1. In the onset.
2. At the end.
3. In the course of these processes.
4. Never.

**? Question 13**

Which “acute phase” protein oxidizes iron and carries copper?

1. Haptoglobin.
2.  $\alpha_2$ -Macroglobulin.
3. Ceruloplasmin.
4. CRP.

**? Question 14**

SP-A and SP-D can bind:

1. Bacterial lysophosphatidylcholine.
2. Bacterial LPSs, fungal glucan, and mannose residues.
3. Carbohydrates on the surface of many microbes.
4. Fc receptors.

**? Question 15**

CRP can bind:

1. Bacterial lysophosphatidylcholine.
2. Bacterial LPSs, fungal glucan, and mannose residues.
3. Carbohydrates on the surface of many microbes.
4. Antigen-binding sites.

**? Question 16**

The polymerization of SAA may lead to:

1. Ichthyosis vulgaris.
2. Amyloidosis.
3. Chronic neuralgia.
4. Acute pneumonia.

### 3.4 The Complement System

#### Definitions

The *complement* is a series of serum proteins capable of activating and destroying with no specificity a wide variety of bacteria, fungi, protozoans, viruses, tumor cells, and own cells.

3

This mechanism of innate immunity was discovered by 1919 Nobel Laureate J. Bordet.

The complement system consists of about 40 functionally linked serum and membrane-bound proteins, which are synthesized in the liver and small and large intestine. During fetal life, complement proteins are made from the sixth week. The complement proteins are as follows:

1. Complement's components designated by letter C (C1–C9)
2. Subcomponents of the 1C (C1q, C1r, and C1s)
3. Fragments of the activated complement such as C2a, C3a, C3b, etc.
4. Factors of the alternative pathway (B, D, and P)
5. Enzymes like convertases
6. Membrane attack complex C5b6789...9 (MAC)
7. Complement inhibitors and inactivators
8. Complement receptors

Concerning C2a and C2b, there are two approaches to their nomenclature. In the first case, the fragment that takes part in the formation of classical pathway C3 convertase is called C2a, whereas in the second case, it is in contrast.

Complement genes are located on many chromosomes, not only on chromosome 6 (Class III HLA genes).

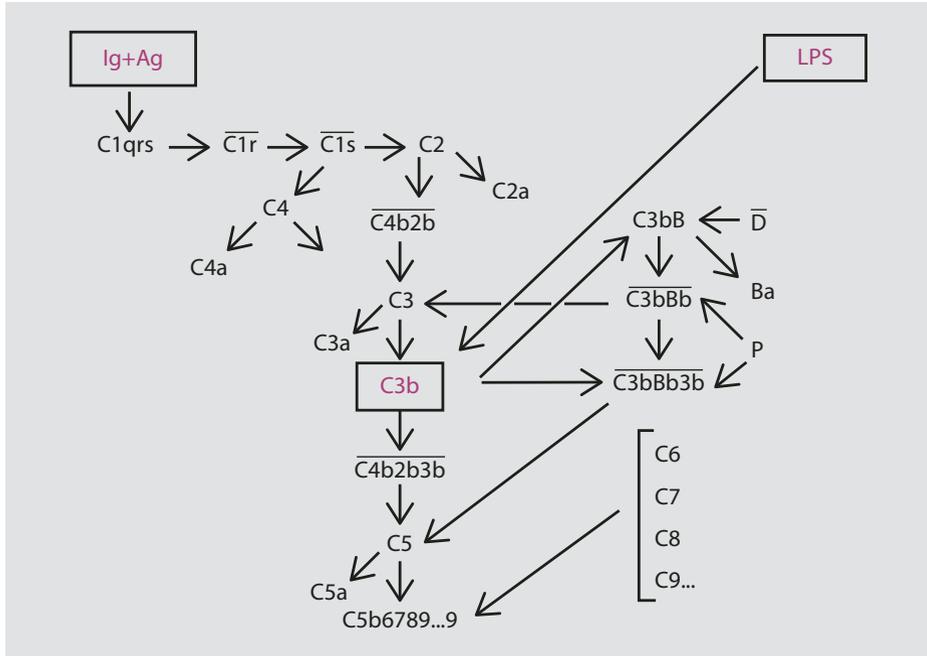
**From a clinical point of view,** deficiencies of the complement components are associated with high susceptibility to pyogenic infections, especially *Neisseria meningitidis*. Besides, defects of C1, C2, and C4–C8 may lead to autoimmune disorders like *systemic lupus erythematosus (SLE)*.

The complement can constitute protein cascades when each activated component catalyzes the activation of the next components and eventually causes the formation of the membrane attack complex (MAC). The consequences of the complement cascade are the lysis of target cells, amplification of inflammation and phagocytosis, and the participation in completing soluble immune complexes, which include an antigen, antibody, and complement fragment.

There are several pathways of complement activation (see ■ Fig. 3.2).

## 3.4 • The Complement System

- Classical pathway (on the left)
  - Lectin subpathway
  - CRP subpathway
- Alternative pathway (on the right)



■ Fig. 3.2 Complement cascade

### 3.4.1 Classical Pathway

The classical pathway is activated by immune complexes. Conformational change in an immunoglobulin molecule bound to an antigen leads to disclosure of the site for binding C1. The C1 consists of six subcomponents C1q of a tulip-like structure including a globular head for attaching an antibody, two C1r and two C1s. When the antibody attached to a particle of interest (e.g., bacterial cell surface or viral envelope) binds to C1q, C1r generates an active enzyme, C1 esterase. Substrates for the enzyme C1s are C2 and C4 that are cleaved into some fragments, C2a/C2b and C4a/C4b, respectively. C4b and C2b constitute a new enzyme, C4b2b, known as the *classical pathway C3 convertase*, which acts on C3. As a rule, during following events, a smaller fragment (–a) will exert powerful biological qualities outside the cascade, whereas a larger fragment (–b) will participate in the formation of new molecules of the complement cascade.

Next, C3 is cleaved into C3a and C3b. C4b, C2b, and C3b form the enzyme C4b2b3b, known as the *classical pathway C5 convertase*, which splits C5 into C5a and C5b, a component of the membrane attack complex, C5b6789...9 (MAC). The MAC makes holes in the membrane of a target cell, leading to its osmotic lysis.

Antibody-independent subpathways, lectin and CRP subpathways, proceed as described above, but soluble C-type lectins and CRP initiate the activation instead.

### 3.4.2 Alternative Pathway

For the alternative activation pathway, constant availability of C3b is required because this pathway does not rely on antigen-binding antibodies. Bacterial lipopolysaccharides may protect C3b due to the downregulation of natural C3b inhibitors (factor I and factor H). Free C3b binds factor B to produce C3bB, a substrate of a circulating enzyme, and factor D, which cleaves B into Ba and Bb. Bb binds to C3b to give the enzyme C3bBb, the *alternative pathway C3 convertase*, stabilized by factor P (properdin), and then the new enzyme C3bBb3b, the *alternative pathway C5 convertase*, is constituted. The subsequent events are analogous to the classical pathway.

During both the classical pathway and alternative pathway of complement activation, a variety of biologically active fragments are formed (see ■ Table 3.1).

■ Table 3.1 Biological qualities of activated complement fragments

Fragment	Qualities
C2a	Being kinin, it causes pain due to irritation of nerve endings
C3a	Exhibits <i>anaphylactic activity</i> <sup>a</sup> Is present in acylation-stimulating protein's form, which upregulates synthesis of adipocytes and skin fibroblasts
C3b	Takes part in the opsonization to facilitate phagocytosis Recruits more factors B, D, and P for the alternative pathway
C4a	Exerts <i>anaphylactic activity</i> <sup>a</sup> Causes edema
C5a	Exhibits <i>anaphylactic activity</i> <sup>a</sup> Has a potency to cell destruction
C5b	Initiates the formation of MAC
Factor P (properdin)	Stabilizes the C3bBb convertase Has direct antibacterial effect
MAC	Lyses target cells

<sup>a</sup>*Anaphylactic activity* is the ability to trigger degranulation of endothelial cells, mast cells, and phagocytes, which produces the local inflammatory response due to migration of leukocytes, increase in permeability of blood capillaries, and contraction of smooth muscle cells like bronchospasm

### 3.4.3 Regulatory Proteins of the Complement

---

The complement typically exerts its activity locally, on the microbial membranes, and therefore must be well-controlled. If complement activation proceeds at the systemic level and is directed at self-cells, the anaphylactoid shock might develop.

Complement activity is controlled by a short-term life span of activated fragments and a variety of inhibitors and inactivators: C1 inhibitor, factor I, factor H, C4-binding protein, protein S, carboxypeptidase N, etc.

*C1 inhibitor (C1 esterase)* blocks the enzymatic activity of C1qrs that limits, in particular, the formation of C4a, a potent odemagenous factor.

**From a clinical viewpoint,** the mutation in C1NH on 11q12.1 is a form of immunodeficiency (C1 inhibitor deficiency, *hereditary angioedema*) characterized by progressive edema of deep tissues, especially dangerous in a case of laryngeal and intestinal edema.

*CD59 (protectin)* and *CD55 (decay-accelerating factor, DAF)* compete with MAC for insertion into the cell membrane and prevent lysis of cells.

**From a clinical viewpoint,** mutations in CD59 on 11p13 and CD55 on 1q32.2 lead to hemolysis, *hemolytic anemia*, and *paroxysmal nocturnal hemoglobinuria*.

*Factor H* modulates the cleavage of C3 convertase, C3bBb, through factor I and DAF. Factor H exhibits its protective action only regarding self-cells and self-surfaces but not on the surfaces of microbes.

**From a clinical viewpoint,** mutations or single nucleotide polymorphisms in factor H on 1q31.3 often result in the group of pathology, in particular, age-related *macular degeneration*, *glomerulonephritis*, and *renal failure*.

### 3.4.4 Complement Receptors

---

There are some complement receptors: CR1 (CD35), CR2 (CD21), CR3 (CD11b/CD18), CR4 (CD11c/CD18), and C5aR (CD88). All complement receptors bind to C3 or fragments of C4 on the pathogen surface, but they have distinct functions.

CR1 is expressed by erythrocytes, which carry immune complexes to the liver and spleen for degradation. Complement receptors CR1, CR3, and CR4 take part in opsonization, and CR2 is a part of B-cell coreceptor and the receptor for *Epstein-Barr virus (EBV)*.

#### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

C3b and C5a are:

1. Complement's components.
2. Fragments of the activated complement.
3. Complement's regulatory proteins.
4. Complement's receptors.

**? Question 2**

B and P are:

1. Complement's regulatory proteins.
2. Factors of the alternative pathway.
3. Subcomponents of C1.
4. Fragments of the activated complement.

**? Question 3**

Lectin subpathway is related to:

1. Alternative pathway of complement activation.
2. Antibody-dependent pathway.
3. Properdin-dependent pathway.
4. Classical pathway.

**? Question 4**

C5b6789...9 is:

1. Membrane attack complex.
2. Classical pathway C3 convertase.
3. Factor of the alternative pathway.
4. Alternative pathway C5 convertase.

**? Question 5**

C2a causes:

1. Edema.
2. Lysis of target cells.
3. Pain due to nerve ending irritation.
4. Target cell activation.

**? Question 6**

Factor P (properdin):

1. Exhibits anaphylactic effects.
2. Takes part in the opsonization.
3. Stabilizes the C3Bb convertase.
4. Destroys target cells.

**? Question 7**

CD35 is:

1. CR2.
2. CR4.
3. CR1.
4. CR3.

**? Question 8**

C4b2b3b is:

1. Classical pathway C5 convertase.
2. Alternative pathway C5 convertase.

### 3.4 • The Complement System

3. Alternative pathway C3 convertase.
4. Classical pathway C3 convertase.

#### ? Question 9

C3bBb is:

1. Classical pathway C3 convertase.
2. Alternative pathway C3 convertase.
3. Alternative pathway C5 convertase.
4. Classical pathway C5 convertase.

#### ? Question 10

The complement system was discovered by:

1. E.E. Metchnikoff.
2. J. Bordet.
3. P. Ehrlich.
4. F.M. Burnet.

#### ? Question 11

C3b:

1. Exhibits anaphylactic effects.
2. Stabilizes the C3Bb convertase.
3. Takes part in the opsonization.
4. Lyses target cells.

#### ? Question 12

Substrates for enzyme C1s are:

1. C2 and C4.
2. C3 and C5.
3. C6 and C7.
4. Factor B and factor P.

#### ? Question 13

The complement does not contribute to:

1. Inflammation.
2. Opsonization.
3. Apoptosis.
4. Completing soluble immune complexes.

#### ? Question 14

Hereditary angioedema is caused by:

1. Deficiency of C3.
2. Deficiency of C1 inhibitor (C1 esterase).
3. Deficiency of factor H.
4. Deficiency of protectin and decay-accelerating factor (DAF).

**? Question 15**

Elevated susceptibility to *Neisseria meningitidis* is linked to:

1. Deficiency of complement components.
2. Deficiency of factor H.
3. Deficiency of C1 inhibitor (C1 esterase).
4. Deficiency of  $\alpha_1$ -antitrypsin.

**? Question 16**

This complement receptor does not participate in opsonization:

1. CR4.
2. CR3.
3. CR1.
4. CR2.

### 3.5 Phagocytosis

---

#### Definitions

*Phagocytosis* is the ability of specialized cells, phagocytes (mainly neutrophils and macrophages), to internalize objects of “non-self,” “former self,” and even “self” and destroy them.

*Incomplete phagocytosis* is the inability of phagocytes (mainly macrophages) to destroy internalized pathogens when these pathogens persist and can spread throughout the body.

*Autophagy* is the physiological and in rare cases pathologic phagocytosis and disassembly of own needless cells to recycle cellular components.

*Phagocytosis* is a mechanism of innate immunity, which is characterized by ingesting microbes, various cells, and particles by (1) professional phagocytes (mainly neutrophils and macrophages), which have specialized sensing receptors, and (2) nonprofessional phagocytes (e.g., endotheliocytes, etc). This phenomenon was discovered by 1908 Nobel Laureate E.E. Metchnikoff, a well-known Russian researcher.

When a site of infection or inflammation occurs, neutrophils very rapidly move to the site. The process of their directed movement is called *chemotaxis*, which is the first phase of phagocytosis. In comparison to neutrophils, macrophages migrate slower and exert their phagocytic qualities more slowly too.

There are four phases of phagocytosis:

1. Chemotaxis
2. Opsonization and adherence
3. Endocytosis and cytotoxicity
4. Degradation of pathogen and exocytosis

■ Fig. 3.3 Endocytosis and formation of phagosome



Phagocytes detect chemical gradients of chemoattractant such as IL8 (CXCL8), IL1, activated complement fragments, leukotriene B4 (LTB4), etc. due to a variety of special receptors on the cell surface. During chemotaxis, phagocytes change their shape becoming asymmetrical with leading front and tail.

Other receptors on the cell surface allow phagocytes to recognize opsonins (e.g., CRP, MBL, C3b, etc.), which coat microbes and particles (*opsonization*), so that phagocytes can adhere to an ingesting microbe (*adherence*) lightly. Antibody opsonization is a contribution of phagocytosis to adaptive immunity.

*Endocytosis* of the ingesting microbes (see ■ Fig. 3.3) is always accompanied by NADPH oxidase's activation and a "respiratory burst," which lead to *cytotoxicity effects* mediated by the oxygen- dependent system. *Reactive oxygen species (ROS)* are extremely toxic to microbes, but they may result in significant damage to self-cells and cell structures. There is such a phenomenon as *oxidative stress*, which is fatal for neutrophils themselves.

1. Generation of *superoxide anion*:  

$$\text{O}_2 + \text{electron} \rightarrow \text{O}_2^-$$
2. Generation of *singlet oxygen*:  

$$\text{H}_2\text{O}_2 + \text{OCl}^- \rightarrow {}^1\text{O}_2 + \text{H}_2\text{O} + \text{Cl}^-$$
3. Generation of *hydroperoxyl*:  

$$\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{OH}^- + \text{OH}$$
4. Generation of *hydrogen peroxide*:  

$$\text{H}_2\text{O} + \text{O}_2^- + \text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2$$
5. *Myeloperoxidase-H<sub>2</sub>O<sub>2</sub>-halogen* system:  

$$\text{H}_2\text{O}_2 + 2\text{Cl}^- + \text{H}^+ \rightarrow \text{H}_2\text{O} + \text{Cl}_2 + \text{OH}^-$$
6. *Nitric oxide cycle*:  

$$\text{NO}_3^- + 2 \text{ electrons} \rightarrow \text{NO}_2^- + \text{electron} \rightarrow \text{NO}^- + \text{O}_2 \rightarrow$$

$$\text{NO}_2^- + \text{H}_2\text{O} \rightarrow \text{NO}_3^-$$

The other cytotoxicity system of phagocytes is the oxygen-independent system, which includes *lysozyme*, *lactoferrin*,  *$\alpha$ -defensins*, etc. and takes part in the degranulation of phagocytes to kill microbes in cooperation with ROS.

Lysozyme splits 1,4- $\beta$ -linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a bacterial cell wall peptidoglycan.

Lactoferrin, an iron-binding glycoprotein, is capable of large binding quantities of iron and so competing with microbes for iron. In addition, lactoferrin binds bacterial lipopolysaccharide (LPS).

$\alpha$ -Defensins have a polar topology with spatially separated charged and hydrophobic regions, which allows them to insert into microbial phospholipid membranes.

Endocytosed microbes fall in phagosomes and then phagolysosomes where final *degradation* of them with the participation of acid hydrolases takes place. After that, microbial debris is *exocytosed*.

**From a clinical point of view,** there are many primary immunodeficiencies associated with phagocytic defects.

*Chronic granulomatous disease (CGD)* occurs in babies, and it is characterized by abscesses of the skin, lymph nodes, and liver caused by opportunistic bacteria. In 80% of cases, it is X-linked immunodeficiency with the location of mutation in CYBB gene on Xp21.1-p11.4, which encodes p22phox for phagocyte oxidase that leads to the inability to generate ROS.

*Leukocyte adhesion deficiencies (LAD)* occur in babies and toddlers, and this disorder is characterized by repeated bacterial and fungal infections, intestinal or perianal fistulae, delayed wound healing, and high leukocytosis. Type 1 LAD is associated with CD18 deficiency (mutation on 21q22.3), type 2 LAD is linked to disorder to synthesize sialylated Lewis<sup>x</sup> (mutation on 11p11.2), and type 3 LAD is caused by the mutation in FERMT3 gene (on 11q13.1).

*Neutrophil G6PD deficiency* arises in male babies with a defect of G6PD gene (Xq28), which encodes G6PD's synthesis that is required for the proper function of hexose monophosphate shunt in the NADPH system. Same symptoms characterize the disorder like chronic granulomatous disease (CGD) as well as nonspherocytic hemolytic anemia.

*Myeloperoxidase deficiency* is associated with the mutation in myeloperoxidase gene (17q22) and is characterized by recurrent fungal infections.

*Specific granule deficiency* exhibits recurrent bacterial infections and anemia. This defect is caused by the mutation in the lactoferrin gene (14q11.2).

*Chédiak-Higashi syndrome* is characterized by albinism, hepatosplenomegaly, and increased susceptibility to infections. The defect is linked to giant peroxidase-positive cytoplasmic lysosomes and mutation of the lysosomal trafficking regulator gene (1q42.3).

### ■ Quiz

Reading a question, please choose only one right answer.

#### 🔍 Question 1

A cell which cannot phagocyte:

1. Eosinophil.
2. Neutrophil.
3. T cell.
4. Macrophage.

## 3.5 • Phagocytosis

**? Question 2**

Phagocytosis was discovered by:

1. P. Ehrlich.
2. E.E. Metchnikoff.
3. P. Langerhans.
4. R.M. Steinman.

**? Question 3**

Chemotaxis is:

1. Cell-to-cell adherence.
2. Undirected movement of cells.
3. "Respiratory burst"
4. Directed movement of cells.

**? Question 4**

Reactive oxygen species (ROS) are:

1. Toxic oxygen radicals.
2. Lysozyme and lactoferrin.
3.  $\alpha$ -Defensins.
4. Opsonins.

**? Question 5**

Endocytosis and cytotoxicity occur after:

1. Chemotaxis of phagocytes.
2. Opsonization and adherence.
3. Exocytosis of microbial debris.
4. Intracellular degradation of pathogens.

**? Question 6**

In comparison to neutrophils, macrophages migrate to pathogens:

1. Faster.
2. Extremely rapidly.
3. Slower.
4. Equally.

**? Question 7**

Chemoattractants differ from chemokines in:

1. Their high specificity.
2. They do not differ.
3. Their little specificity.
4. They have the equal ability to induce the speed of cell migration.

**? Question 8**

In the course of phagocytosis, the leading front of neutrophils is attributed to:

1. Their asymmetrical shape if neutrophils move.
2. Adherence to an ingesting pathogen.
3. Formation of phagosome.
4. Formation of phagolysosomes.

**? Question 9**

Superoxide anion is related to:

1. Oxygen-independent system of phagocytes.
2. Reactive oxygen species.
3.  $\alpha$ -Defensins.
4. Chemoattractants.

**? Question 10**

Opsonization and adherence occur after:

1. Exocytosis of microbial debris.
2. Chemotaxis.
3. Intracellular degradation of pathogens.
4. Endocytosis and cytotoxicity.

**? Question 11**

IL8 (CXCL8) is simultaneously a chemoattractant and chemokine:

1. No way.
2. Unknown.
3. Yes.
4. Probably.

**? Question 12**

Phagocytosis may contribute to the adaptive immunity:

1. Yes.
2. No.
3. No way.
4. Unknown.

**? Question 13**

Internalized microbes first fall in:

1. Phagolysosomes.
2. Extracellular space.
3. Phagosomes.
4. Nucleus of phagocyte.

**? Question 14**

Type I leukocyte adhesion defect is associated to:

1. CD15 deficiency.
2. CD18 deficiency.
3. Mutation of BTK gene.
4. CD19 deficiency.

**? Question 15**

Lysozyme splits:

1. 1,4- $\beta$ -Linkages in bacterial peptidoglycans.
2. Links between amino acid residues in peptides.
3. Disulfide bonds.
4. Hydrogen links.

**? Question 16**

Kupffer's cells are macrophages in:

1. The kidney.
2. The liver.
3. The lungs.
4. The bone.

### 3.6 NETosis

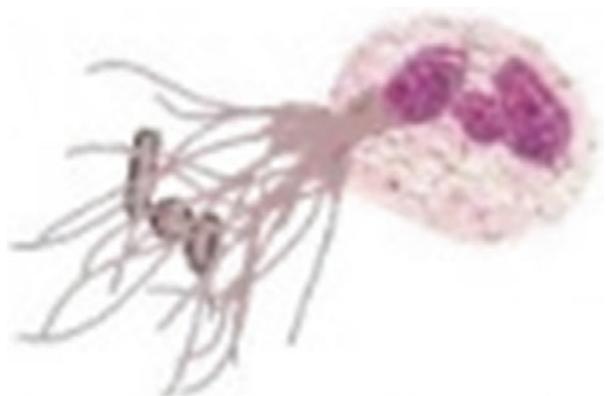
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**Definitions**

*NETosis* is the ability of some cells including *neutrophils*, *eosinophils*, and *mast cells* to extrude chromatin-derived threads in response to pathogens to immobilize them.

NETosis is a novel mechanism of innate immunity, which is employed by neutrophils, eosinophils, and mast cells. The term “NETosis” comes from “neutrophil extracellular trap” (NET). The NET’s loop is composed of chromatin fibers with a diameter of 15–17 nm by which neutrophils, eosinophils, and mast cells catch up and immobilize microbes, whereas there is no essential damage to the host cells. Furthermore, NET formation develops very rapidly and does not lead to the death of the neutrophils (see ■ Fig. 3.4). After that, NETs inactivate pathogens by means of antimicrobial proteins such as neutrophil elastase, cathepsin G, and histones, which have a high affinity for DNA. NETs may be cleaned away by macrophages that engulf and degrade them.

■ Fig. 3.4 NETosis



However, high-resolution scanning electron microscopy has demonstrated that NETs also contain globular protein domains with a diameter of 25 nm. They aggregate into more massive threads with a diameter of 50 nm and greater that allow them to promote the formation of blood clots.

Recent investigations suggest that NETs may play an essential role in both defense against infections and the pathogenesis of thrombotic disorders. Some researchers study the participation of NETs in the pathogenesis of autoimmune diseases.

**From a clinical viewpoint,** if NETosis threads are more extensive than 50 nm in diameter, they may promote the formation of endovascular clots.

### 3.7 Natural Cytotoxicity

#### Definitions

*Interferons (IFNs)* are the cytokines, which can induce cells to resist viral replication and certain tumor cell proliferation that is called *natural cytostasis*.

*Natural killer (NK) cells* are a type of innate lymphoid cells (ILCs), which destroy via *apoptosis* virus-infected cells, own aging and impaired cells, and certain tumor cells that is called *natural cytotoxicity*.

*Apoptosis* is a form of cell death in which the cell-like NK cell activates via some extrinsic or intrinsic pathways an intracellular death program associated to *caspase cascade*. *Bcl-2 proteins* inhibit apoptosis.

*Necrosis* is an accidental cell death when cells swell and lose the membrane integrity before shutting down and realizing their intracellular contents into extracellular space.

#### 3.7.1 Interferons (IFNs)

Interferons (IFNs) are cytokines, which are secreted by a variety of cell types under the influence of some inducers such as viral envelope glycoproteins, CpG DNA, dsRNA, and other pathogen-associated molecular patterns (PAMPs) as well as tumor-associated molecular patterns (TAMPs).

Type I interferons, *IFN $\alpha$*  and *IFN $\beta$* , are secreted by many cell types including lymphocytes (NK cells, B cells, and T cells), macrophages, dendritic cells, fibroblasts, endothelial cells, osteoblasts, and others and exhibit powerful antiviral and antitumor effects. They also stimulate NK cells to elicit an antiviral and antitumor response. Besides, *IFN $\alpha$*  acts as a pyrogenic and painful factor by affecting thermosensitive neurons in the hypothalamus that causes fever and pain. On the other hand, *IFN $\alpha$*  interacts with the  $\mu$ -opioid receptor to act as an analgesic. To affect a target cell, *IFN $\alpha$*  and *IFN $\beta$*  use the CD118 molecule as the receptor.

Type II *IFN $\gamma$*  is produced by activated T cells, B cells, and NK cells and also exerts some antiviral and antitumor effects, but these effects are generally weaker than those of *IFN $\alpha$*  and *IFN $\beta$*  exhibit. However, *IFN $\gamma$*  is a potent immunoregulatory cytokine, which

functions in a synergic manner with  $\text{TNF}\alpha$ ,  $\text{TNF}\beta$ , IL1, IL6, and other cytokines and chemokines, and is extremely important for almost all immune processes. The CD119 molecule is the receptor for  $\text{IFN}\gamma$ .

To date, several effector pathways of the IFN-mediated antiviral response have been described. IFN-inducible enzymes inside a cell infected by a virus individually block viral transcription, degrade viral RNA, inhibit translation, and control all steps of viral replication. As a result, *natural cytostasis* takes place.

The main effector pathways of the IFN-mediated antiviral response are as follows:

- Protein kinase R (PKR)
- 2',5'-Oligoadenylate-synthetase (OAS) with the participation of ribonuclease L (RNase L)
- Mx protein GTPases

Protein kinase R (PKR), an RNA-dependent protein kinase, inhibits the translation of viral proteins through phosphorylation of protein synthesis initiation factor  $\text{eIF-2}\alpha$ .

2',5'-Oligoadenylate-synthetase (OAS) mediates RNA degradation. For this process, the second enzyme, ribonuclease L (RNase L), is required, which becomes activated by binding 2–5A oligonucleotides.

Mx protein GTPases appear to target viral nucleocapsids and inhibit RNA synthesis. The Mx protein alone is sufficient to block the replication of the virus in the absence of any other  $\text{IFN}\alpha$ -/ $\text{IFN}\beta$ -inducible enzymes, but the Mx protein is not induced by  $\text{IFN}\gamma$ .

Among such cytokines as IL2, IL12, IL15, IL18, and CCL5 (RANTES), IFNs are also powerful activators of NK cells.

**From a clinical viewpoint,** mutations in *IFN $\alpha$ 2* and *IFN $\beta$ 1* genes (9p21.3) are linked to increased susceptibility to viral infections of the respiratory tract and the predisposition to some types of cancer.

Mutations in *IFN $\gamma$*  gene (12q15) promote increased susceptibility to viral and bacterial including mycobacterial infections and parasitic invasions and enable potentiating some autoimmune disorders.

The mutation in *IFNGR1* (*CD119*) on 6q23.3 (*immunodeficiency 17A/a7B*) leads to SCID-like symptoms, defects of phagocytosis, and development of severe inflammation in response to *Mycobacteria* and different pathogens.

### 3.7.2 NK Cells

*Natural killer (NK) cells*, large granular lymphocytes (LGL), *CD16<sup>hi</sup>CD56<sup>lo</sup>*, refer to group 1 of innate lymphoid cells (ILCs) and are essential for innate immunity (see ■ Fig. 3.5). NK cells develop from common lymphoid progenitor cells in the bone marrow and become self-tolerant by recognition of Class I HLA gene products during T-cell-like education. Mature NK cells migrate to the thymus, liver, and secondary organs of the immune system where they differentiate into functionally distinct mature

■ **Fig. 3.5** Large granular lymphocyte (LGL)



■ **Table 3.2** Some killer-inhibitory and killer-activating NK-cell receptors

Receptor's designation	Receptor's name	Ligand	Function
KIR	Killer immunoglobulin-like receptor	Class I HLA molecules	Inhibitory or activating
NKG2/CD94	Natural killer (lectin-like) receptor G2/CD94	HLA-E	Inhibitory or activating
NCR	Natural cytotoxicity receptor	Viral hemagglutinin	Activating
LILR	Leukocyte immunoglobulin-like receptor	Class I HLA molecules	Inhibitory
KLRG1	Killer lectin-like receptor G1	Cadherins	Inhibitory

NK-cell subsets. The important chemokines for NK-cell migration are CCL3 (MIP-1 $\alpha$ ), CCL4 (MIP-1 $\beta$ ), CCL5 (RANTES), and CX3CL1 (fractalkine). NK cells express two types of receptors, which deliver either activating or inhibitory signals to target cells. NK-cell inhibitory receptors (IRs) and activating receptors (ARs) are a very complex group of receptors, which recruit opposing signaling motifs to upregulate or downregulate the activation of NK cells to trigger apoptosis in target cells (see ■ Table 3.2). IRs signal through intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs), located in the cytoplasmic tail of these receptors. Conversely, most ARs signal through immunoreceptor tyrosine-based activating motifs (ITAMs), localized in AR-associated molecules, whereas other ARs use an alternative signaling mechanism.

NK cells can lyse any target cells without prior sensitization. The target cells for NK cells are cells infected by viruses, tumor cells, and aging and injured cells of the body. The missing “self”-hypothesis suggests that IRs recognize Class I HLA molecules and cancel apoptosis in a target cell, whereas if Class I HLA molecules are not expressed on the surface of the cell, ARs trigger apoptosis in the cell.

NK cells may be activated following the loss of Class I HLA molecules on the surface of abnormal cells or the appearance of stressed cells in response to infection or malignant transformation. The activation of NK cells leads to a release of perforin piercing target cell membrane, running granzyme through pore inside the cell, and launching caspase cascade, which induces *apoptosis* in the target cell. It is *natural cytotoxicity*. However, NK cells are engaged by cytotoxic CD8 + T cells at the effector phase of the adaptive immune response. Furthermore, recently it was demonstrated that NK cells could exhibit several features attributed to adaptive T cells and B cells such as the expansion and contraction of subsets, increased longevity, and formation of immunological memory, which is characterized by a more powerful response upon secondary exposure to the same antigen.

**From a clinical viewpoint,** there are some primary immunodeficiencies linked to the disorder of NK-cell activity. For example, selective functional deficiency of NK cells called *immunodeficiency 20* is caused by the rare mutation in CD16 (FCGR3A) gene on 1q23.3. Natural, spontaneous cytotoxicity is decreased, but antibody-dependent cellular cytotoxicity (ADCC) is preserved. Patients suffer from recurrent viral infections such as *Epstein-Barr virus (EBV)*, *herpes zoster virus (HZV)* and other herpesviruses, and *human papillomavirus (HPV)*.

### 3.7.3 NK-Cell Subsets

$CD16^{hi}CD56^{lo}$  NK cell is the main subpopulation of NK cells. However, there are some other NK-cell subsets, which should be named.

The  $CD16^{lo}CD56^{hi}$  NK-cell subset predominates in the secondary lymphoid organs of the immune system and the liver and is probably responsible for tolerance to symbiotic flora, opportunistic microbes in the resting state, and food proteins.

$CD56^{bright}$  uterine NK cells (*uNK cells*) play an important role in early pregnancy and probably contribute to the successful pregnancy. They lack or have the low cytotoxic ability, which may be due to the presence of ligands for their inhibitory receptors because trophoblast cells downregulate HLA-A and HLA-B expression.

#### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

CD118 is:

1. The receptor to  $IFN\gamma$ .
2. The receptor to  $IFN\alpha$  and  $IFN\beta$ .
3. NK-cell-activating receptor.
4. NK-cell inhibitory receptor.

**? Question 2**

IFN $\gamma$  acts in a synergic manner with:

1. TNF $\alpha$  and TNF $\beta$ .
2. IL4 and IL13.
3. IL10 and TGF $\beta$ .
4. IL3 and IL5.

**? Question 3**

This enzyme does not take part in the IFNs associated degradation of mRNA:

1. Mx protein GTPases.
2. 2',5'-Oligoadenylate-synthetase (OAS).
3. Protein kinase R (PKR).
4. C3bBb3b.

**? Question 4**

CD119 is:

1. The receptor to IFN $\gamma$ .
2. The receptor to IFN $\alpha$  and IFN $\beta$ .
3. NK-cell inhibitory receptor.
4. NK-cell-activating receptor.

**? Question 5**

IFN $\alpha$  refers to:

1. Type II interferon.
2. Type I interferons.
3. Chemokines.
4. Colony-stimulating factors (CSFs).

**? Question 6**

Interferons:

1. Inhibit NK cells.
2. Potentiate cancer progression.
3. Stimulate NK cells.
4. Amplify virus replication.

**? Question 7**

CD16<sup>hi</sup>CD56<sup>lo</sup> NK cells are related to:

1. Group 2 of innate lymphoid cells (ILCs).
2. Dendritic cells (DCs).
3. Group 1 of ILCs.
4. Group 3 of ILCs.

**? Question 8**

NK cells participate in:

1. Antibody-dependent cell cytotoxicity (ADCC).
2. Innate immunity only.
3. Adaptive immunity only.
4. Complement activation.

**? Question 9**

NK cells are capable of:

1. Internalizing pathogens.
2. Inducing apoptosis in virus-infected cells.
3. Activating phagocytosis.
4. Sensing Class II HLA molecules on autologous cells.

**? Question 10**

NK cells express:

1. TCR and BCR.
2. Activating and inhibitory receptors to trigger apoptosis in target cells.
3. Complement receptors.
4. Phagocytic receptors.

**? Question 11**

Functionally, NK cells are close to:

1. Mast cells.
2. T cells.
3. NKT cells.
4. B cells

**? Question 12**

Natural cytotoxicity receptor (NCR) is:

1. Activating receptor.
2. Receptor to  $\text{IFN}\gamma$ .
3. Receptor to  $\text{IFN}\alpha$ .
4. Inhibitory receptor.

**? Question 13**

Leukocyte immunoglobulin-like receptor (LILR) is:

1. Receptor to  $\text{IFN}\gamma$ .
2. Activating receptor.
3. Receptor to  $\text{IFN}\beta$ .
4. Inhibitory receptor.

**? Question 14**

CD16<sup>lo</sup>CD56<sup>hi</sup> NK cells are responsible for:

1. Simple B-cell-mediated immune response.
2. Tolerance to symbiotic bacteria and opportunistic microbes in the steady state and food proteins.
3. Type I hypersensitivity.
4. Type IV hypersensitivity.

**? Question 15**

NK cells may exert:

1. Immunological memory.
2. The ability to phagocytose pathogens.
3. The capacity to activate complement.
4. Tendency to promote tumor cell growth.

**? Question 16**

Ligands for NK-cell inhibitory receptors are:

1. Class II HLA molecules.
2. Integrins.
3. Selectins.
4. Class I HLA molecules.

### 3.8 Inflammasome, Pyroptosis, and Physiological Inflammation

---

#### Definitions

*Simple inflammation* is a type of inflammation, which gets started urgently before adaptive immune responses are progressing up to their effector stage.

*Inflammasome* is a unit of inflammatory process that can be formed due to infectious invaders or tissue injury.

*Pyroptosis* is a cell death form different from apoptosis since it is caused by the plasma membrane rupture and release of DAMPs and pro-inflammatory cytokines into the extracellular space.

*Inflammasome* is a unit of innate immunity that can be formed rapidly in response to infectious invaders and/or tissue injury. The inflammasome is a multiprotein complex expressed in myeloid cells, which activates caspase-1, processes pro-inflammatory cytokines IL1 $\beta$  and IL18, and induces cell pyroptosis, a process of programmed cell death different from apoptosis. The exact composition of an inflammasome depends on the activator, which initiates inflammasome assembly. Such activators are microbe-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) that act through a variety of pattern recognition receptors (PRRs). Among the PRRs, TLRs can detect PAMPs/DAMPs in the extracellular environment and endosome, whereas NLRs and some other PRRs play a crucial role in sensing “patterns” in the intracellular compartments.

The NLRP3 inflammasome (see **■** Fig. 3.6) is the best-studied inflammasome and seems to be activated by many microbes. Structurally, NLRs contain LRR domains, a

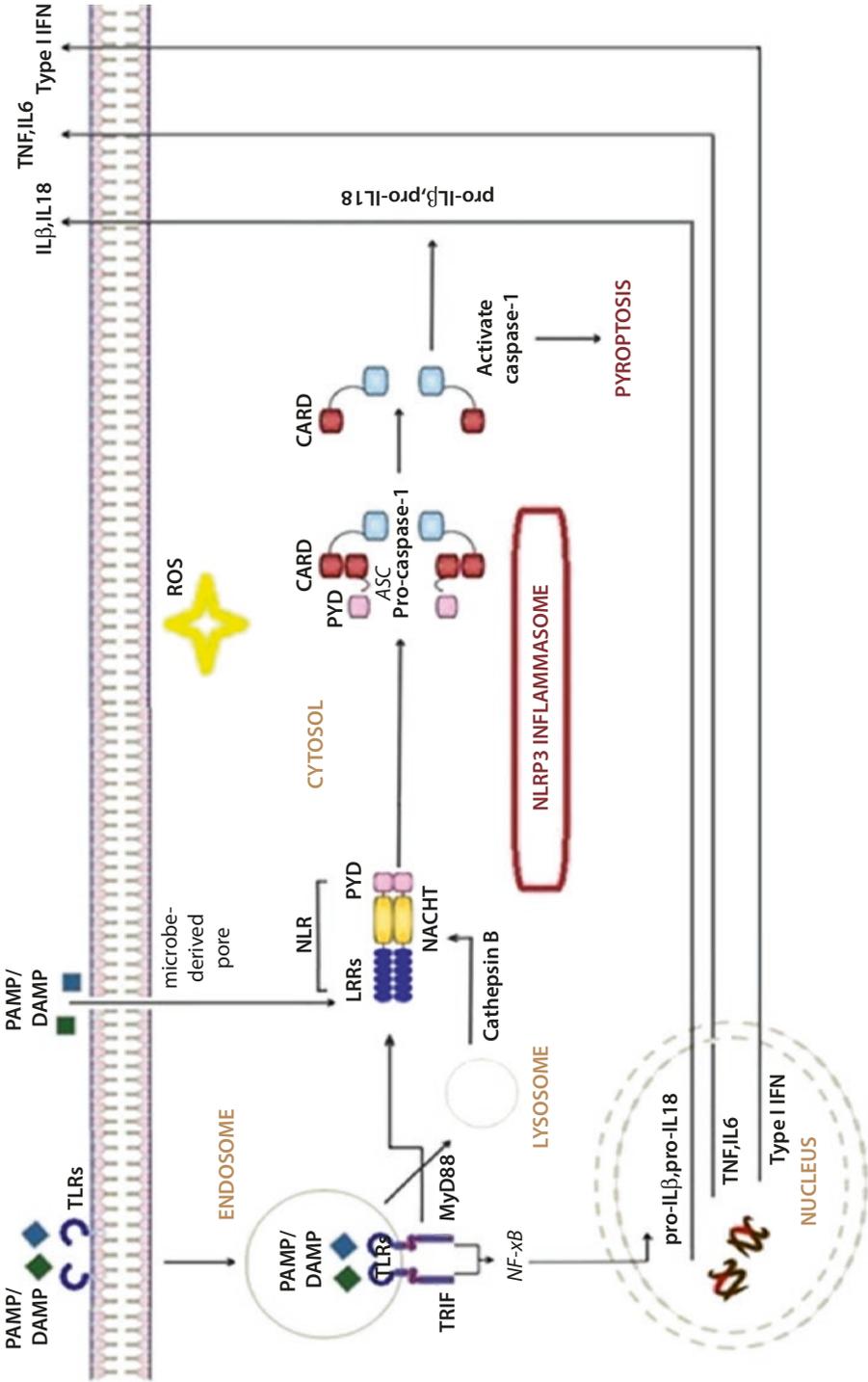


Fig. 3.6 NLRP3 Inflammasome

NACHT domain, and an effector domain, which can be either a pyrin domain (PYD), or caspase recruitment domain (CARD), or another type. The effector pathways associated with the inflammasome act at different levels.

PAMPs/DAMPs are recognized by TLRs expressed on neutrophils and macrophages and then phagocytosed. In phagosome (endosome), PAMPs/DAMPs associated to TLRs trigger TLR signaling pathways, which recruit some adaptor proteins such as MyD88 and TRIF. The received signal activates the transcription factor NF- $\kappa$ B to promote the gene expression of cytokines including TNF, IL6, pro-IL1 $\beta$ , and pro-IL18. Other transcription factors induce the gene expression of type I IFN. After the formation of phagolysosomes, lysosomal components including cathepsin B are released and activate the NLRP3 inflammasome with the participation of adaptor protein ASC. In addition, PAMPs/DAMPs may enter the cell through microbe-derived (e.g., pantexin-1) pores and directly activate the NLRP3 inflammasome. Also, PAMPs/DAMPs induce the production of reactive oxygen species (ROS), which may trigger NLRP3 inflammasome activation.

Upon activation of the inflammasome, caspase-1 processes pro-IL1 $\beta$  and pro-IL18 into their active forms. The subsequent process, *pyroptosis*, also requires the involvement of caspase-1. The term “pyroptosis” comes from the Greek word “pyro” meaning “fire.” As opposed to apoptosis, cell death during pyroptosis is caused by plasma membrane rupture and the release of DAMP such as ATP, DNA, and ASC oligomers and pro-inflammatory cytokines into the extracellular space that recruits more cells for the inflammatory cascade in the tissue. The inflammatory cascade may either prevent or resolve infection or even eliminate the infectious agents.

**From a clinical point of view,** in certain instances, inflammasome overactivation leads to tissue damage. Mutation in NLRP3 gene (1q44), which encodes the protein cryopyrin, leads to *multisystem inflammatory disease*, a form of the congenital autoinflammatory disorder. Also, an excess of pro-inflammatory cytokines may lead to *toxic shock syndrome*.

The formation and activation of inflammasome is the well-regulated process that plays a crucial role in maintaining inflammation at the physiological level. At least two groups of proteins may act as negative regulators of inflammasome assembly and caspase-1-dependent production of pro-inflammatory cytokines: PYD-only proteins (POPs) and CARD-only proteins (COPs). During inflammasome assembly, POPs inhibit the PYD-PYD and PYD-ASC interaction, whereas COPs downregulate the recruitment of ASC and processing of pro-IL1 $\beta$ .

Physiological and pathological inflammatory processes differ from each other (see ■ Table 3.3).

■ **Table 3.3** Physiological and Pathological Inflammation

Feature	Acute physiological	Acute pathological	Chronic pathological
Onset	Fast (minutes, hours)	Fast (hours)	Slow (days)
Duration	Minutes, hours	Hours, days	Weeks, months, years
Participation of cells	Mainly neutrophils and macrophages	Mainly neutrophils and macrophages	Monocytes, macrophages, eosinophils, mast cells, and lymphocytes
Exudation	No	Yes	No
Thrombosis	No	Yes	Maybe
Tissue injury	Uncommon and self-limited	May be severe and progressive	May be severe and progressive
Connective tissue hyperplasia	No	No	Yes
Angiogenesis	No	No	Yes
Feature	Acute physiological	Acute pathological	Chronic pathological
Local and systemic signs	Often subtle	Prominent	Prominent, less prominent or subtle
Requires treatment	No	Yes	Yes
Life-threatening	No	Maybe	Maybe

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

Activators of the inflammasome formation are:

1. "Acute phase" proteins.
2. Fragments of activated complement.
3. PAMPs and other types of "patterns."
4. Chemoattractants.

#### ? Question 2

These activators act through:

1. Pattern recognition receptors (PRRs).
2. TCR.
3. Complement receptors.
4. BCR.

**? Question 3**

These receptors do not refer to PRRs:

1. Toll-like receptors (TLRs).
2. NOD-like receptors (NLRs).
3. RIG-1-like receptors (RLRs).
4. Complement receptors CR1, CR2, CR3, and CR4.

**? Question 4**

The NLRP3 inflammasome produces:

1. Pro-inflammatory cytokines.
2. Chemokines.
3. Neurotransmitters.
4. Hormones.

**? Question 5**

Pyroptosis is induced by:

1. IFN $\gamma$ .
2. Caspase-1.
3. Tyrosine kinase btk.
4. Phospholipase C $\gamma$ 1.

**? Question 6**

The NLRP3 inflammasome can also produce:

1. Chemokines.
2. Transforming growth factor- $\beta$  (TGF $\beta$ ).
3. Type I IFNs.
4. IL35.

**? Question 7**

As contrasted to apoptosis, pyroptosis is linked to:

1. Intracellular death program.
2. Necrosis.
3. Extracellular inflammation.
4. NK-cell cytotoxicity.

**? Question 8**

Acute physiological inflammation is characterized by:

1. Fast onset (minutes, hours).
2. Slow onset (days).
3. Very slow onset (years).
4. Fast onset (hours).

**? Question 9**

Acute pathological inflammation is characterized by the exudation:

1. No way.
2. Yes.
3. Unknown.
4. Probably.

**? Question 10**

Chronic pathological inflammation is characterized by the participation of many cell types:

1. Unknown.
2. Yes.
3. Probably.
4. No.

**? Question 11**

Treatment is not required in the case of:

1. Acute pathological inflammation.
2. Any type of the inflammation.
3. Acute physiological inflammation.
4. Chronic pathological inflammation.

**? Question 12**

Prominent local and systemic signs are described in the case of:

1. Acute pathological inflammation.
2. Chronic pathological inflammation.
3. Acute physiological inflammation.
4. Any type of the inflammation.

**? Question 13**

A pro-inflammatory cytokine is:

1. IL10.
2. TGF $\beta$ .
3. IL1 $\beta$ .
4. IL35.

**? Question 14**

An anti-inflammatory cytokine is:

1. IL18.
2. IL10.
3. IL6.
4. TNF $\alpha$ .

**? Question 15**

Reactive oxygen species (ROS) can trigger the NLRP3 inflammasome activation:

1. Yes.
2. No.
3. Probably.
4. Unknown.

**? Question 16**

An adaptor protein, which transduces signal from TLRs, is:

1. BLNK.
2. SLP76.
3. LAT.
4. MyD88.

**Key Points**

1. Innate immunity required for the first line of defense includes epithelial barriers, “acute phase” reaction, complement activation, phagocytosis and NETosis, natural cytotoxicity by NK cells, and simple inflammation by the development of inflammasomes and pyroptosis. Pattern recognition theory is the paradigm of the innate immunity and can be also an important addition to the clonal selection theory.
2. A wide variety of the innate mechanisms were studied in the past. Nowadays, such events as the formation of various types of inflammasome, pyroptosis, recognition of “molecular patterns,” NETosis, etc. are the fertile field of research.
3. Almost all mechanisms of the innate immunity are not functionally isolated and involved at the effector stage of adaptive immune responses.

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# Adaptive Immune Responses

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**Electronic Supplementary Material** The online version of this chapter ([https://doi.org/10.1007/978-3-030-03323-1\\_4](https://doi.org/10.1007/978-3-030-03323-1_4)) contains supplementary material, which is available to authorized users.

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## Learning Objectives

*Knowledge.* Upon successful completion of the chapter, students should be able to:

1. Name and characterize types of the adaptive immune responses.
2. Describe the stages of adaptive immune responses.
3. Outline the antigen processing.
4. List and describe the signals of recognition in the course of adaptive responses.
5. Draw “dual recognition.”
6. Define costimulatory and coinhibitory molecules.
7. Describe the signal transduction and activation of lymphocytes after the recognition of antigens and other signals.
8. Summarize the T-cell and B-cell clonal expansion.
9. Compare the T-cell and B-cell differentiation.
10. Describe the mechanisms of effector T cells and B cells.
11. Draw the levels of regulation of adaptive responses.
12. Be familiar with the idio/anti-idiotype network.
13. Distinguish between nonadaptive and adaptive regulatory (helper) cells.
14. Describe hepatic and neuroendocrine regulation of the immune processes.
15. Explain the principles of genetic regulation of the immune processes.
16. Describe the targets and mechanisms of immunological tolerance.

*Acquired Skills.* Upon successful completion of the chapter, students should demonstrate the following skills, including:

1. Interpret the knowledge related to adaptive immunity.
2. Critically evaluate the scientific literature about the mechanisms of adaptive responses.
3. Discuss the scientific articles from the current research literature to criticize experimental data and formulation of new hypotheses in basic immunology.
4. Attain a clear perception of the presented immunology definitions expressed orally and in written form.
5. Formulate the introduced immunology terms.
6. Correctly answer quiz questions.

*Attitude and Professional Behaviors.* Students should be able to:

1. Have the readiness to be hardworking.
2. Behave professionally at all times.
3. Recognize the importance of studying and demonstrate a commitment.

## 4.1 Introduction

---

The second line of body defense against external invaders and internal enemies is adaptive immune responses. The adaptive responses are categorized into four types depending on the participation of T cells and B cells, the formation of memory cells type, and end effector mechanisms. There are the criticism and discussion related to the classical

T helper 1/T helper 2 paradigm and the description of new regulatory nonadaptive and adaptive lymphocyte subsets such as Tfh, Tfr, Th9, Th17, and Th22. The reader can also find the information on the hepatic, metabolic, neuroendocrine, and genetic regulation of the adaptive immunity and mechanisms of maintenance immune tolerance. Clinical comments include the description of primary immunodeficiencies on the base of mutations in genes crucial for the adaptive immunity and clinical significance of end effector mechanisms in deviating from the healthy state conditions, e.g., immune inflammation.

## 4

## 4.2 Pathways and Stages of Adaptive Immune Responses

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Adaptive immune responses include antigen-specific defense mechanisms and may take days or weeks to develop. These responses are orchestrated by the complex interactions and activities of a large number of various cell types involved in the processes. There are four pathways of adaptive immune responses depending on the type of pathogen.

*Simple B-cell-mediated responses* to T-independent antigens and/or “patterns” belonging to extracellularly located pathogens proceed with the involvement of *naive B cells* but with no aid from helper T cells. However, such a response leads to the production of IgM only, whereas other isotypes of the antibodies and the long-term immunological memory do not occur.

*Advanced B-cell-mediated responses* to antigens derived from extracellularly located pathogens proceed with the participation of *naive B cells* and type 2 helper CD4+ T (Th2) cells/follicular helper T (Tfh) cells. Such a response results in the maturation of plasma cells, antibody switching (subsequently IgM, IgG, IgA, and even IgE) by control of type 1 helper CD4+ T (Th1) cells in part, and producing long-term (lifelong) immunological memory to the antigen due to memory B cells and a relatively short-term memory due to long-lived plasma cells.

The *HLA II pathway of the T-cell-mediated response* involves *naive CD4+ T cells* that result in their clonal expansion and differentiation to effector CD4+ T cells with inflammatory potential. In the beginning, the same cells are type 1 helper CD4+ T cells. This immune response is required to eliminate some intracellularly located exogenous antigens by immune inflammation. Long-term (lifelong) immunological memory CD4+ T cells occur in any case.

The *HLA I pathway of the T-cell-mediated response* engages *naive cytotoxic CD8+ T cells*, which are activated with the aid of type 1 helper CD4+ T cells. Subsequently, CD8+ T-cell clonal expansion proceeds, and the cells mature until they become effector cytotoxic CD8+ T cells in order to take part in the elimination of such endogenous (intracellular) pathogens like viruses. It is achieved by apoptosis in those target cells, which contain the viruses. Lifelong memory CD8+ T cells are always available.

There are six stages of adaptive immune responses, as follows:

1. Antigen endocytosis, processing, and loading on Class I HLA or Class II HLA molecules for presentation to lymphocytes
2. “Dual recognition” of the antigen/HLA I molecule or antigen/HLA II molecule complexes and simultaneous recognition of two other signals from costimulatory/coinhibitory molecules and cytokines
3. Signal transduction (signaling) and activation of lymphocyte clones
4. Clonal expansion, or proliferation, of the lymphocytes
5. Differentiation of the lymphocyte effector and memory cells
6. Effector activity of the lymphocytes and engaged cells

Antigen-presenting cells (APCs), including dendritic cells, macrophages, and B cells, have crucial roles in the adaptive immune responses. They encounter a native antigen or several native antigens in the site of infection, endocytose them, then accumulate, and carry to the secondary organs of the immune system.

Macrophages phagocytose large intracellularly located antigenic objects, e.g., infected cells, fungi, protozoans, etc., while dendritic cells pinocytose viruses and proteins, and B cells internalize microbial toxins.

### 4.3 Antigen Processing

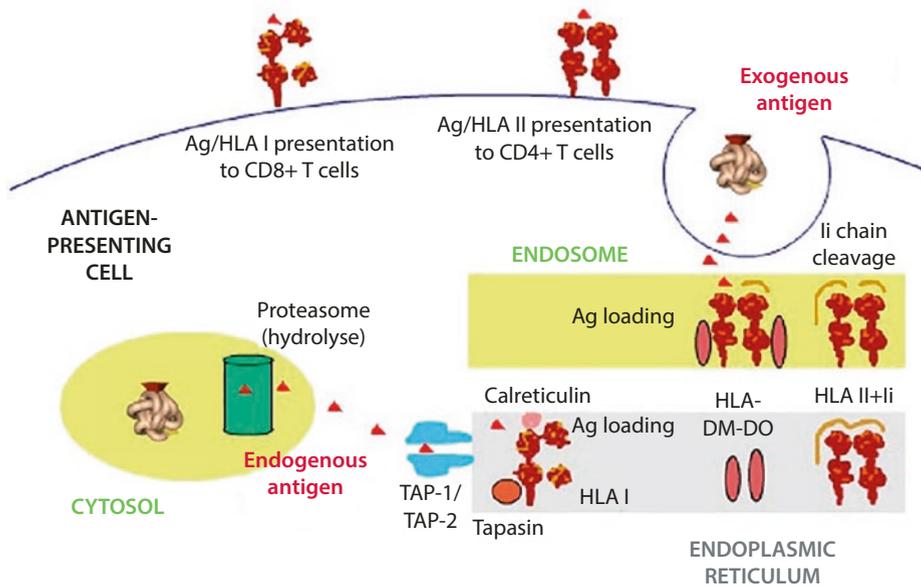
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#### Definitions

*Antigen processing* is an event, which proceeds in antigen-presenting cells in order to disassemble native antigens and prepare them for presentation to lymphocytes.

*Chaperones* are proteins, which are required for both the covalent folding or unfolding and the assembly or disassembly of other macromolecular substances such as HLA molecules.

Antigen-presenting cells (APCs) encounter native antigens, endocytose, and process them. A subsequent event, *processing*, implies the disassembly of native antigens and complexation of single epitopes with Class I and Class II HLA molecules inside the antigen-presenting cell. The processed antigen becomes an immunogen, i.e., an immunologically active substance. There are two pathways of antigen processing, depending on the antigen type (see ■ Fig. 4.1).



■ Fig. 4.1 Antigen processing

*Exogenous antigens* are presented in association with Class II HLA molecules to naive CD4+ T cells (the *HLA II pathway*). In the beginning, a native antigen is endocytosed and degraded by proteolytic enzymes in endosomes or lysosomes. At the same time, Class II HLA molecules in association with a chaperone, *invariant (Ii) chain*, are generated from gene transcription and translation and are assembled in the endoplasmic reticulum. The Ii chain, including the CLIP region, is required for the protection of the HLA molecule groove until the antigen is ligated. Next, the HLA II molecule/Ii chain complex is transported through the Golgi apparatus into the endosomes where the Ii chain is lost and other chaperones, HLA-DM and HLA-DO, are complexed to the Class II HLA molecule to stabilize their intermediate empty before uploading an antigenic epitope. After that, the HLA molecule binds the antigenic epitope, and this complex is transported to the surface of antigen-presenting cell.

*Endogenous and intracellularly disposed microbe-derived antigens such as viral antigens* are loaded onto the Class I HLA molecules (the *HLA I pathway*) to be presented to naive CD8+ T cells. In contrast to those exogenous antigens, a cytoplasmic antigen is translocated to the cytosol to be cleft by a large proteolytic complex, proteasome, which is composed of three components (LMP-2, LMP-7, and MECL-1). After that, antigenic peptides are transported through the TAP-1/TAP-2 “tunnel” into the endoplasmic reticulum. At the same time, Class I HLA molecules are assembled in the endoplasmic reticulum, and their grooves are thereafter protected by chaperones, calnexin and calreticulin, which stabilize the empty groove before uploading the antigenic epitope. The HLA molecules are also complexed to another chaperone, tapasin. Finally, the Class I HLA molecule/antigenic epitope complex is transported to the surface of the antigen-presenting cell.

**From a clinical viewpoint,** mutations in TAP-1/TAP-2 genes (on chromosome 6p21.3) lead to a SCID, *type I deficiency of Class I HLA molecules (type 1 bare lymphocyte syndrome)*.

**■ Quiz**

Reading a question, please choose only one right answer.

**? Question 1**

Antigen processing is:

1. Preparation of native antigens for presentation to dendritic cells.
2. Assembly and disassembly of HLA molecules.
3. Preparation of native antigens for presentation to lymphocytes.
4. Folding of proteins.

**? Question 2**

Antigen processing proceeds:

1. In eosinophils.
2. In T cells.
3. In antigen-presenting cells.
4. In neurons.

**? Question 3**

Extracellular antigen processing takes place:

1. In endoplasmic reticulum.
2. In cytosol.
3. In nucleus.
4. In endosomes.

**? Question 4**

Intracellular antigen processing proceeds:

1. In endoplasmic reticulum.
2. In cytosol.
3. In endosomes.
4. In Golgi apparatus.

**? Question 5**

Intracellular antigen processing depends:

1. On Class II HLA molecules.
2. On Class I HLA molecules.
3. On enzymes in endosomes.
4. On phospholipase C $\gamma$ 1.

**? Question 6**

Extracellular antigen processing depends:

1. On Class II HLA molecules.
2. On Class I HLA molecules.
3. On enzymes in proteasome.
4. On phospholipase C $\gamma$ 2.

**? Question 7**

Chaperones are responsible:

1. For cleavage of polypeptide chains.
2. For signaling.
3. For folding or unfolding of other proteins.
4. For thymic positive selection of T cells.

**? Question 8**

Ii chain is important in the course of:

1. Extracellular antigen processing.
2. Intracellular antigen processing.
3. Phagocytosis.
4. Formation of inflammasomes.

**? Question 9**

Calnexin is important in the course of:

1. Extracellular antigen processing.
2. Intracellular antigen processing.
3. Thymic negative selection of T cells.
4. Pyroptosis.

**? Question 10**

Antigenic epitope is loaded on:

1. On  $\beta_2$  microglobulin.
2. Groove of HLA molecule.
3. HLA-DO.
4. HLA-DM.

**? Question 11**

Class I HLA/antigen complex is transported:

1. Into the nucleus.
2. Into endosomes.
3. On the surface of antigen-presenting cell.
4. In the proteasome.

**? Question 12**

Class II HLA/antigen complex is transported:

1. Into the nucleus.
2. In the proteasome.
3. On the surface of antigen-presenting cell.
4. In Golgi apparatus.

**? Question 13**

Class I HLA/antigen complex is presented:

1. Macrophages.
2. Dendritic cells.
3. Lymphocytes.
4. Mast cells.

## 4.4 • “Dual Recognition” and Other Signals

**?** Question 14

Class II HLA/antigen complex is presented:

1. Neutrophils.
2. Lymphocytes.
3. Macrophages.
4. Dendritic cells.

**?** Question 15

Mutations in TAP-1/TAP-2 genes result in:

1. Type 1 bare lymphocyte syndrome.
2. Chronic granulomatous disease.
3. Common variable immunodeficiency (CVID).
4. Type I diabetes mellitus.

**?** Question 16

Class I HLA/antigen complex is presented:

1. To naive CD4+ T cells.
2. To memory T cells.
3. To memory B cells.
4. To naive CD8+ T cells.

**4.4 “Dual Recognition” and Other Signals**

---

**Definitions**

*Recognition* is the interaction of lymphocytic receptors with their counter-receptors on antigen-presenting cells.

The *first signal* for the recognition is provided with the *dual recognition* that is the interaction of antigenic receptors and coreceptors on lymphocytes with HLA molecules (“self”) and antigens (“non-self”) on antigen-presenting cells.

The *second signal* is the stimulus provided with the *costimulatory/coinhibitory molecules*, which are expressed on both lymphocytes and antigen-presenting cells. The *third signal* is the stimulus from cytokines.

During the simple B-cell-mediated immune response, BCR recognizes and binds antigens directly, in a cross-linking manner. The V domains are exposed on the surface of B cell, whereas C domains remain inserted in the membrane of the B cell. According to the classical model, at least two individual BCRs have to be bound by a polyvalent antigen for B-cell activation. Conversely, short monomer soluble antigens promote B cells to unresponsiveness.

During an advanced B-cell-mediated response and T-cell-mediated responses, BCR and TCR do not recognize and bind antigens directly but instead recognize epitopes, short peptide fragments of antigens, which are uploaded onto Class I and Class II HLA molecules on the surfaces of antigen-presenting cells. The space between any antigen-presenting cell and lymphocyte is termed the “immunological synapse.” The formation

of the immunological synapse takes about 6 h. Recognition of the epitope/HLA molecule complex is called *dual recognition*, i.e., the simultaneous recognition of “non-self” and “self” (*signal 1*). TCR or BCR recognizes the antigen, whereas a coreceptor recognizes HLA molecules.

This phenomenon was discovered by 1996 Nobel Laureates P.C. Doherty and R.M. Zinkernagel.

However, the other types of signals are required as those subsequent events might proceed. Both antigen-presenting cells and lymphocytes express costimulatory and coinhibitory molecules, B7 family molecules like B7–1 (CD80) and B7–2 (CD86), on antigen-presenting cells, and CD28 and CTLA-4 (CD152) on lymphocytes (*signal 2*). In the T-cell-mediated responses, CD28 transmits a positive signal to T cells, whereas CTLA-4 (CD152) makes a negative signal. In the advanced B-cell-mediated response, the signals from these costimulatory molecules are opposite; therefore they are coinhibitory molecules. The positive signals induce upregulation of the IL2 receptor, which enhanced IL2 mRNA translation and IL2 secretion, and subsequent T-cell or B-cell growth. In addition, many different families of costimulatory and coinhibitory molecules are described over the past decade (see ■ Table 4.1). CD40L is required in the process of “help” naive CD8+ T cells from type 1 helper CD4+ T cells. In the course of the recognition, cells are also closely connected to each other by some adhesion molecules such as ICAMs and LFA-1.

Nowadays, lots of costimulatory/coinhibitory molecules are discovered (s. ■ Table 4.1).

Cytokines, chemokines, and their receptors make another required signal. In total, there are two pathways of recognition, *type 1 helper-dependent pathway* and *type 2 helper T cell/follicular helper T-cell-dependent pathway*. Cytokine effects are received by T cells and B cells from antigen-presenting cells, NK cells, mast cells, and a variety of cell types. The back signal such as IFN $\gamma$  secretion maintains the Class I HLA and Class II HLA molecules expression on the surfaces of antigen-presenting cells. Cytokines (*signal 3*), which have upregulation effects on lymphocytes, may be divided into two groups depending on the type of the recognition pathway: IL12, IL18, TNF $\alpha$  and TNF $\beta$  for the Th1 pathway, and IL4, IL6, and IL21 for the Th2/Tfh pathway.

All types of recognition are provided in a series of figures below (■ Figs. 4.2, 4.3, 4.4, and 4.5).

As you can see, a variety of various costimulatory/coinhibitory molecules, cytokines, and adhesion molecules take part in recognition. Thus, there are three types of required signals for recognition:

1. Specific interaction between antigen/HLA molecules and BCR/coreceptor or TCR/coreceptors
2. Additional interplay between many costimulatory molecules
3. Additional interaction between some cytokine, chemokines, and their receptors

■ **Table 4.1** Some costimulatory/coinhibitory molecules

Molecules	Expression	Ligands
CD28	CD4+ T cells (+) CD8+ T cells (+) B cells (–)	B7–1, B7–2
CTLA-4 (cytotoxic T lymphocyte antigen-4) (CD152)	CD4+ T cells (–) CD8+ T cells (–) B cells (+)	B7–1, B7–2
ICOS (inducible T-cell costimulator) (CD278)	CD4+ T cells (+) CD8+ T cells (+)	B7-H2
BTLA (B and T lymphocyte attenuator)	CD4+ T cells (–) CD8+ T cells (–) B cells (–)	HVEM (herpes virus entry mediator)
CD40L (CD154)	CD4+ T cells (+)	CD40
CD40	CD8+ T cells (+) B cells (+)	CD40L (CD154)
OX40 (CD134)	CD4+ T cells (+) CD8+ T cells (+)	OX40L (CD252)
CD30	CD4+ T cells (+) CD8+ T cells (+) B cells (+)	CD30L (CD153), TRAFs
CD27	CD4+ T cells (+) B cells (+)	CD70
SLAM (signaling lymphocytic activation molecule) (CD150)	CD4+ T cells (±) CD8+ T cells (±)	SLAM (signaling lymphocytic activation molecule) (CD150)
2B4 (CD244)	CD4+ T cells (+) CD8+ T cells (+)	CD48
CD48	CD4+ T cells (+) CD8+ T cells (+) B cells (+)	2B4 (CD244)

(+) costimulation, (–) coinhibition

#### 4.4.1 Type 1 Helper T-Cell-Dependent Pathways

#### 4.4.2 Type 2 Helper T Cells/Follicular Helper T-Cell-Dependent Pathway

After recognition, signals are transmitted inside the cells, which causes B-cell and T-cell activation, clonal expansion, and production of specific effector molecules (antibodies) and effector CD4+ T cells and CD8+ T cells.

4

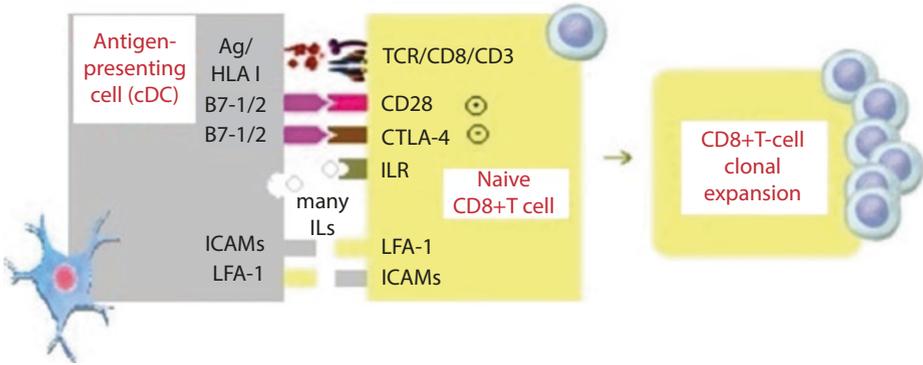


Fig. 4.2 Recognition by naive CD4+ T Cells (Th1)

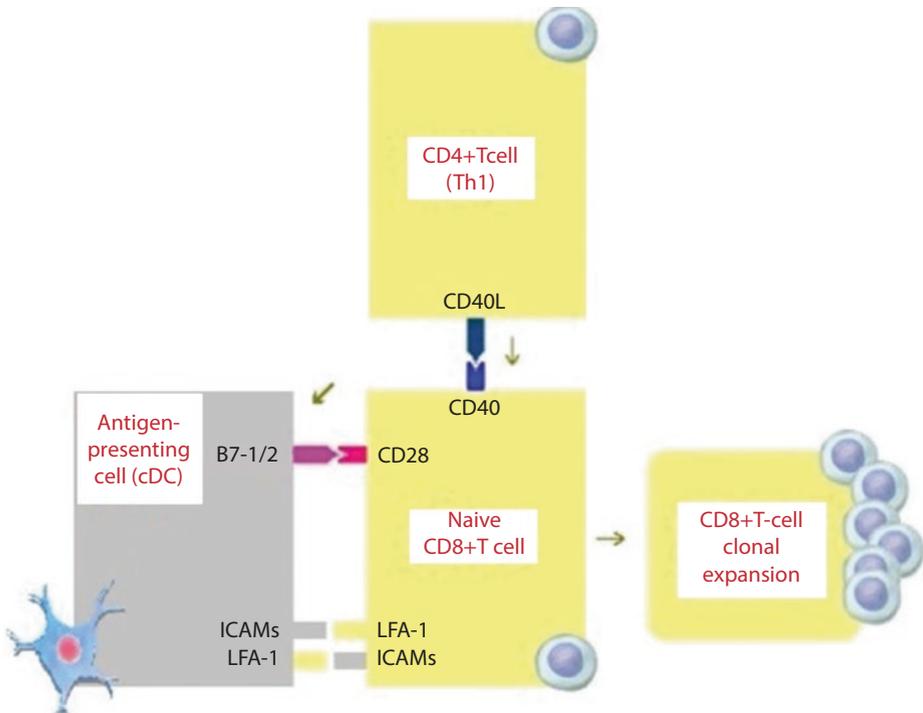
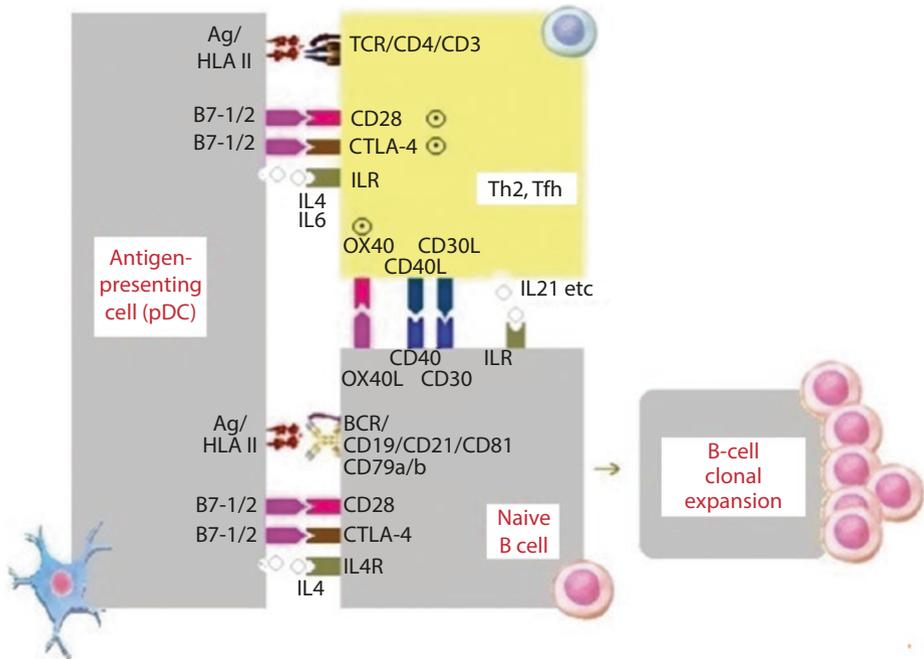
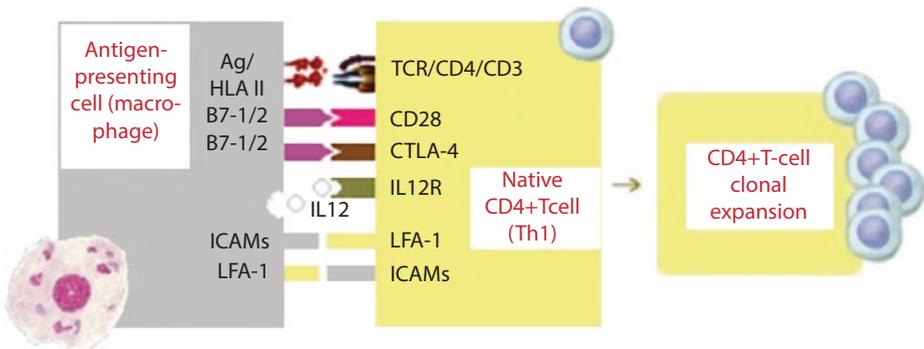


Fig. 4.3 Recognition by naive CD8+ T Cells

## 4.4 • “Dual Recognition” and Other Signals



■ Fig. 4.4 “Help” naive CD8+ T cells from CD4+ T cells (Th1)



■ Fig. 4.5 Recognition by naive B cell with “help” from CD4+ T cells (Th2/Tfh)

**■ Quiz**

Reading a question, please choose only one right answer.

**? Question 1**

In the course of recognition, signal 1 is:

1. The signal from costimulatory molecules.
2. The signal from cytokines and chemokines.
3. The signal from HLA/antigen complex.
4. The signal from coinhibitory molecules.

**? Question 2**

In the course of recognition, signal 2 is:

1. The signal from phagocytes.
2. The signal from cytokines and chemokines.
3. The signal from HLA/antigen complex.
4. The signal from costimulatory/coinhibitory molecules.

**? Question 3**

In the course of recognition, signal 3 is:

1. The signal from costimulatory molecules.
2. The signal from cytokines and chemokines.
3. The signal from HLA/antigen complex.
4. The signal from coinhibitory molecules.

**? Question 4**

“Dual recognition” was discovered by:

1. P.C. Doherty and R.M. Zinkernagel.
2. S. Tonegawa.
3. B.A. Beutler, J.A. Hoffmann, and R.M. Steinman.
4. C.A. Janaway.

**? Question 5**

“Dual recognition” is:

1. Simultaneous recognition of some cytokine stimuli.
2. Simultaneous recognition of the HLA molecule and antigen.
3. Recognition of signals from costimulatory/coinhibitory molecules.
4. Interaction between hormones and their receptors.

**? Question 6**

“Immunological synapse” is:

1. The space between two neurons, which secrete neurotransmitters.
2. The space between phagocyte and opsonized bacterium.
3. The space between antigen-presenting cell and lymphocyte.
4. Extracellular space around lymphocytes.

## 4.4 • “Dual Recognition” and Other Signals

**? Question 7**

Signal from CD28 upregulates:

1. Activation of T cells.
2. Maturation of dendritic cells.
3. Activation of B cells.
4. Positive selection of B cells in the bone marrow.

**? Question 8**

Signal from CTLA-4 (CD152) upregulates:

1. Activation of T cells.
2. Activation of B cells.
3. Maturation of dendritic cells.
4. Positive selection of T cells in the thymus.

**? Question 9**

IL12 upregulates:

1. Activation of B cells.
2. Proliferation of memory B cells.
3. Activation of type 2 helper T cells.
4. Activation of T cells.

**? Question 10**

IL4 upregulates:

1. Activation of B cells.
2. Proliferation of both subsets of memory T cells.
3. Activation of type 1 helper T cells.
4. Activation of type 17 helper T cells.

**? Question 11**

Type 1 helper T cell helps CD8+ T cells by:

1. Secretion of IL21.
2. Secretion of IL13.
3. Expression of CD40 on CD8+ T cells.
4. Expression of CTLA-4 (CD152) on CD8+ T cells.

**? Question 12**

Type 1 helper T cell also helps CD8+ T cells by:

1. Upregulation of expression of B7 molecules.
2. Upregulation of CTLA-4 expression.
3. Downregulation of secretion of IFN $\gamma$ .
4. Upregulation of secretion of IL4.

**? Question 13**

Coreceptor CD4+ takes part in:

1. Recognition by B cells.
2. Apoptosis in target cells.
3. Recognition by CD4+ T cells.
4. Recognition by phagocytes.

**? Question 14**

Coreceptor CD19+ participates in:

1. Recognition by CD8+ T cells.
2. Apoptosis in target cells.
3. Recognition by B cells.
4. Recognition by CD4+ T cells.

**? Question 15**

ICAMs provide with:

1. Signaling.
2. Closely connection between cells, which take part in recognition.
3. Downregulation of IL2 secretion.
4. Upregulation of IL2 secretion.

**? Question 16**

TNF $\alpha$  and TNF $\beta$  are important for:

1. Th2 pathway.
2. Tfh pathway.
3. Th1 pathway.
4. Th2/Tfh pathway.

## 4.5 Signaling and Lymphocyte Activation

---

### Definitions

*Tyrosine kinases*, a subclass of protein kinase, are the enzymes, which attach a phosphate group from ATP to tyrosine, an amino acid in a protein sequence.

*Adaptor proteins* are the proteins often with no enzymatic activity that facilitate the formation of a variety of large signaling complexes through the particular protein-protein interactions.

*Calcineurin* is the calcium-dependent serine-threonine phosphatase, which upregulates NFAT, a transcription factor.

*Transcription factors* are the proteins, which regulate the gene expression in the particular cell at the right time and in the right amount.

*Signaling pathways* are the directions of signal transduction from cell surface receptor/ligand complexes inside the cell to result in certain effects, e.g., cell mitoses or cytokine synthesis. The signaling pathways are controlled by various signaling molecules such as tyrosine kinases, adapter proteins, etc.

Normally, during adaptive immune responses, about 0.0001–0.001% of the body's T cells and B cells are activated. In signaling, couples of ligands/receptors and ligands/coreceptors, tyrosine kinases, adaptor proteins, a set of different enzymes, and transcription factors are involved to achieve the specific functional outcome of the activated cell.

All signals must be transduced and amplified inside the lymphocyte by a series of reactions to be efficient. Through cell-cell contact and a range of received powerful cytokines, the lymphocyte activates and promotes the clonal expansion of other lymphocytes and secretion of other cytokines as well as the growth of monocytes and granulocytes.

A typical consequence of the signal transduction from antigen-recognizing receptor/coreceptor/accessory molecules is as follows:

- Activation of the tyrosine kinase cascade unique for each pair of ligand/receptor
- Engagement of the adaptor proteins
- Switching on of primary signaling pathways
- Gene transcription

TCR and BCR have very short cytoplasmic tails that lack enzymatic activity. For T-cell and B-cell signaling, CD3 and Ig $\alpha$ /Ig $\beta$  possess ITAM (immunoreceptor tyrosine-based activating motif) whose tyrosine residues are phosphorylated by tyrosine kinases of different families. During *T-cell signaling*, tyrosine kinases (Lck, Zap70, Itk, etc.) are involved in the activation process, which through adaptor proteins (LAT, SLP76, NCK, etc.) switch on some primary signaling pathways leading to the IL2 gene transcription and cell mitoses.

During the *first signaling pathway*, a membrane phospholipid, phosphatidylinositol diphosphate, is hydrolyzed by ATP-dependent phospholipase C $\gamma$ 1 (PLC $\gamma$ 1) to produce inositol triphosphate and diacylglycerol. These products cause an increase in intracellular Ca<sup>2+</sup> and activation of the protein kinase C, respectively. The protein kinase C has many of the downstream effects, including activation of nuclear transcription factors (NF- $\kappa$ B, etc.). Calcium-dependent protein calcineurin upregulates the transcription factor NFAT, which requires for the IL2 gene expression.

The *second signaling pathway* involves the activation of phospholipase A2 via diacylglycerol and Rac/Pho GTPases through the exchange factor Vav and plays an important role in T-cell proliferation and differentiation.

*B-cell signaling*, as well as signaling from CD40, IL21R, and BAFFR, parallels the pathways described for the TCR, but the B-cell pathways have their peculiarities and are multiple. Cytoplasmic tyrosine kinase, Syk, Lyn, Btk, and others; adaptor proteins BLNK, GRB2, etc.; and enzyme PLC $\gamma$ 2 are essential for BCR signaling.

**From a clinical viewpoint,** mutations in the associated receptors/signaling molecules lead to some primary immunodeficiencies (see ■ Table 4.2).

**Table 4.2** Molecular anomalies of receptors/signaling molecules and primary immunodeficiencies

Signaling molecule	Chromosome	Immunodeficiency
CD3 $\gamma$ or CD3 $\delta$	11q23.3	Immunodeficiency 17/19 (deficiency of CD3 $\gamma$ or CD3 $\delta$ )
CD19	16p11.2	Common variable immunodeficiency (CVID)
CD21	1q32.2	
CD81	11p15.5	
Zap70	2q11.2	Immunodeficiency 48 (deficiency of CD8)
Btk	Xq21.3–q22	X-linked agammaglobulinemia (Bruton's syndrome)

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

In the course of signaling, the tyrosine kinase cascade for each pair of ligand/receptor is:

1. Repeated.
2. The same.
3. Unique.
4. Unknown.

#### ? Question 2

Which tyrosine kinases do not part in T-cell signaling?

1. Btk.
2. Syk.
3. p56<sup>lck</sup>.
4. Zap70.

#### ? Question 3

The enzyme, which does not participate in T-cell signaling pathways:

1. Phospholipase C $\gamma$ 2.
2. Protein kinase C.
3. Lipoxygenase.
4. Phospholipase C $\gamma$ 1.

#### ? Question 4

Adapter proteins are:

1. Signaling molecules.
2. Enzymes.
3. Nuclear transcription factors.
4. Tyrosine kinases.

**? Question 5**

Signaling consequence is:

1. One-step process.
2. Four-step process.
3. Two-step process.
4. Ten-step process.

**? Question 6**

In the course of a signaling pathway, the protein kinase C is activated by:

1. Phosphatidylinositol diphosphate.
2. Tyrosine kinase Zap70.
3. Inositol triphosphate and diacylglycerol.
4. Nuclear transcription factors.

**? Question 7**

In the course of a signaling pathway, the phospholipase A2 is activated by:

1. Phosphatidylinositol diphosphate.
2. Nuclear transcription factors.
3. Diacylglycerol.
4. Tyrosine kinase Syk.

**? Question 8**

Activation of nuclear transcription factors is caused by:

1. Protein kinase C.
2. Phospholipase C $\gamma$ 1.
3. Tyrosine kinase p56<sup>lck</sup>.
4. Adapter protein LAT.

**? Question 9**

In comparison with T-cell signaling, B-cell signaling:

1. Is identical.
2. Has some peculiarities.
3. Unlike.
4. Unknown.

**? Question 10**

X-linked agammaglobulinemia (Bruton's syndrome) is caused by mutation in:

1. Zap70 gene.
2. Tyrosine kinase p56<sup>lck</sup> gene.
3. BTK gene.
4. CD19 gene.

**? Question 11**

Immunodeficiency 48 (deficiency of CD8) is caused by mutation in:

1. Zap70 gene.
2. BTK gene.
3. CD19 gene.
4. Tyrosine kinase p56<sup>lck</sup> gene.

**4** **?** **Question 12**

Common variable immunodeficiency (CVID) may be caused by mutation in:

1. Zap70 gene.
2. Tyrosine kinase p56<sup>lck</sup> gene.
3. BLNK gene.
4. CD19 gene.

**?** **Question 13**

Common variable immunodeficiency (CVID) may be caused by mutation in:

1. Zap70 gene.
2. BTK gene.
3. CD16 gene.
4. CD81 gene.

**?** **Question 14**

This adapter protein is important for B-cell signaling:

1. LAT.
2. SLP76.
3. BLNK.
4. ASC.

**?** **Question 15**

During T-cell signaling, activation of nuclear transcription factors in T cells can result in:

1. IL2 gene expression.
2. Antibody synthesis.
3. Formation of plasma cells.
4. Somatic hypermutations.

**?** **Question 16**

Common variable immunodeficiency (CVID) may be caused by mutation in:

1. CD21 gene.
2. Zap70 gene.
3. BLNK gene.
4. BTK gene.

## 4.6 Clonal Expansion

---

**Definitions**

*Clonal expansion* is the multiple proliferation of activated daughter lymphocytes with identical antigenic receptor derived from the same parent lymphocyte.

According to Burnet's clonal selection theory, if a random antigen invades the body, its pre-existing T-cell clone and B-cell clone are involved and produce a large number of identical lymphocytes directed against the antigen. Clonal expansion is the process by which daughter cells arise from a parent cell. Lymphocytes multiply from just a few to millions during the clonal expansion, and the process gives the adaptive immune system its extraordinary might and specificity. *IL2* is the major universal growth factor for T cells and B cells during the clonal expansion.

The *clonal expansion of T cells* proceeds in paracortical zones of the lymph nodes, periarterial lymphatic sheaths of the spleen, and parafollicular zones of the MALT. Former naive T cells are stimulated into proliferation and turned into lymphoblasts, which then become effector lymphocytes and memory cells. Whereas the CD8+ T cells generate the large clone rapidly, the CD4+ T cells proliferate to the abundant number of the identical clonal CD4+ T cells more slowly. The clonal expansion is upregulated by a variety of cytokines such as IL2, IL9, IL12, IL15, IL18, IFN $\gamma$ , TNF $\alpha$ , etc. and costimulatory molecules like CD28. On the contrary, CTLA-4 counts for much in the downregulation of the T-cell clonal proliferation.

Antigen-recognizing naive B cells are in follicles of the lymph nodes, spleen, and MALT. Primary follicles due to the *B-cell clonal expansion* develop into secondary follicles. The B cells are stimulated into mitosis during which they grow into centroblasts and then as centrocytes depending on a stage of the process in the secondary follicle's center. B-cell growth is upregulated by a variety of cytokines such as IL2, IL4, IL5, IL6, IL10, IL13, IL14, IL21, BAFF, IFN $\gamma$ , TNF $\alpha$ , etc. and some costimulatory molecules: CD30, CD40, and OX40L. As opposed to T-cell proliferation, during the B-cell clonal expansion, the cells may temporarily lose their BCR and undergo so-called somatic hypermutations as a maneuver for high-affinity BCR selection. Typically, an increase in affinity occurs in the positive selection of B cells with high-affinity BCRs and the negative selection of B cells with low-affinity BCRs. The B cells with the highest affinity will then be selected to differentiate into plasma cells, which produce antibodies. Simultaneously, long-lived plasma cells and lifelong memory B cells are constituting. They contribute to the rapid advanced B-cell-mediated immune response upon reinfection by the same pathogen.

**From a clinical point of view,** when clonal expansion occurs, a physician may palpate the swollen lymph nodes in the neck or other accessible areas. However, the swollen lymph nodes may be a sign of severe pathology.

#### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

In adaptive immune responses, the process by which daughter cells arise from a parent cell is:

1. Signaling.
2. Recognition.
3. Clonal expansion.
4. Effector activity.

**? Question 2**

Clonal expansion of T cells proceeds in:

1. Paracortical zones of the lymph nodes.
2. Follicles of MALT.
3. Secondary follicles of the lymph nodes.
4. The thymus.

**4****? Question 3**

Clonal expansion of B cells proceeds in:

1. Paracortical zones of the lymph nodes.
2. Secondary follicles of the lymph nodes.
3. Periarteriolar lymphatic sheaths of the spleen.
4. Parafollicular zones of the MALT.

**? Question 4**

CD4+ T cells proliferate faster than CD8+ T cells:

1. No.
2. Unknown.
3. Yes.
4. Research is in progress.

**? Question 5**

CD8+ T cells proliferate faster than CD4+ T cells:

1. Yes.
2. Unknown.
3. No way.
4. Research is in progress.

**? Question 6**

Signal from CD28 upregulates T-cell expansion:

1. No.
2. Yes.
3. Research is in progress.
4. Unknown.

**? Question 7**

Signal from CTLA-4 (CD152) upregulates T-cell expansion:

1. Research is in progress.
2. Unknown.
3. No.
4. Yes.

## 4.6 • Clonal Expansion

**? Question 8**

Signal from CD28 upregulates B-cell expansion:

1. No.
2. Yes.
3. Research is in progress.
4. Unknown.

**? Question 9**

Signal from CTLA-4 (CD152) downregulates B-cell expansion:

1. Research is in progress.
2. Unknown.
3. No.
4. Yes.

**? Question 10**

BCR is:

1. B-cell signaling molecule.
2. B-cell receptor.
3. B-clone recognition.
4. B-cell gene rearrangement.

**? Question 11**

The main growth factor for the proliferation of lymphocytes in the course of adaptive immune responses is:

1. IL1.
2. IL10.
3. IL2.
4. TGF $\beta$ .

**? Question 12**

This cytokine is important during B-cell clonal expansion:

1. IL21.
2. IL35.
3. IL12.
4. IL15.

**? Question 13**

A tool for the high-affinity BCR selection is:

1. Antigen processing.
2. "Dual recognition."
3. Somatic hypermutations.
4. Pyroptosis.

**?** Question 14

During clonal expansion, B cells with the low-affinity BCRs undergo:

1. Necrosis.
2. Apoptosis.
3. Pyroptosis.
4. NETosis.

4

**?** Question 15

B cells with the high-affinity BCRs are ready to differentiation into:

1. Plasma cells.
2. Dendritic cells.
3. NK cells.
4. Innate lymphoid cells (ILC).

**?** Question 16

Antibodies are produced by:

1. Mast cells.
2. Plasma cells.
3. Interdigitating dendritic cells.
4. T cells.

## 4.7 Lymphocyte Differentiation in the Course of Immune Responses

---

### Definitions

*Memory cells* are the lymphocytes, which arise at the end of primary adaptive immune responses to antigen. As contrasted to naive lymphocytes, they respond rapidly on the second exposure to the antigen.

*Central memory T (TCM) cells, effector memory T (TEM) cells, memory B cells, and long-lived plasma cells* are the different types of memory cells.

Lymphocyte differentiation is closely connected to cell clonal expansion. Cloned daughter lymphocytes differentiate into either effector cells or memory cells.

*T-cell differentiation* proceeds in the same zones of the secondary organs as T-cell clonal expansion. Activated by the same cytokines and costimulatory molecules, naive T cells mature up to inflammatory CD4<sup>+</sup> T lymphocytes, cytotoxic CD8<sup>+</sup> T cells, memory CD4<sup>+</sup> T cells, and memory CD8<sup>+</sup> T cells. During maturation, T cells progressively change their phenotype but not morphological shape. They enhance the expression of some cell adhesion molecules, LFA-1 and CD2, lose L-selectins, and begin to display VLA-4. As a result, in a typical infectious case by the 10th day, they all become effector and memory T cells with the same affinity of the TCR for the causative antigen. CD8<sup>+</sup> T cells turn into cytotoxic T cells, whereas CD4<sup>+</sup> T cells continue to differentiate into both subsets of helper T cells and inflammatory T cells.

■ **Table 4.3** Cytokine regulation of antibody switching

Helper T cells	Type 2 helper T cell		Type 1 helper T cell		Type 2 helper T cell	
Secreted cytokine	IL4, IL5, IL6, IL13	IL4, IL6, IL10	IFN $\gamma$ , TNF	TNF	IL5, IL6, IL10	IL4, IL13
Isotypes of the antibodies	IgM	IgG1	IgG2, IgG3	IgA	IgA	IgE, IgG4

*B-cell differentiation* starts out in the same follicles of the secondary organs as B-cell clonal expansion proceeds, but subsequently, it partially relocates to the bone marrow and somewhat to the MALT. As opposed to T-cell differentiation, for B-cell maturation, specific morphological changes are characteristic. In the beginning, B cells develop into immunoblasts, and next, they become lymphoplasmacytoid cells. Plasma cells are the terminal stage of B-cell differentiation. The plasma cells, effector cells of B-cell-mediated responses, contribute to antibody synthesis. Within 1–2 days in a typical infectious case, IgM synthesis occurs. However, due to the somatic hypermutations by the 5th–7th days of the case, the high-affinity B cells are generated in the plasma cells, which can produce IgG. Plasma cells produce enormous amounts of antibodies, about 2000 molecules per second. Antibody switching is dependent on cytokines and costimulatory molecules (see ■ Table 4.3).

In addition, CD40L-CD40 interaction is strictly required for both antibody switching and the formation of memory B cells.

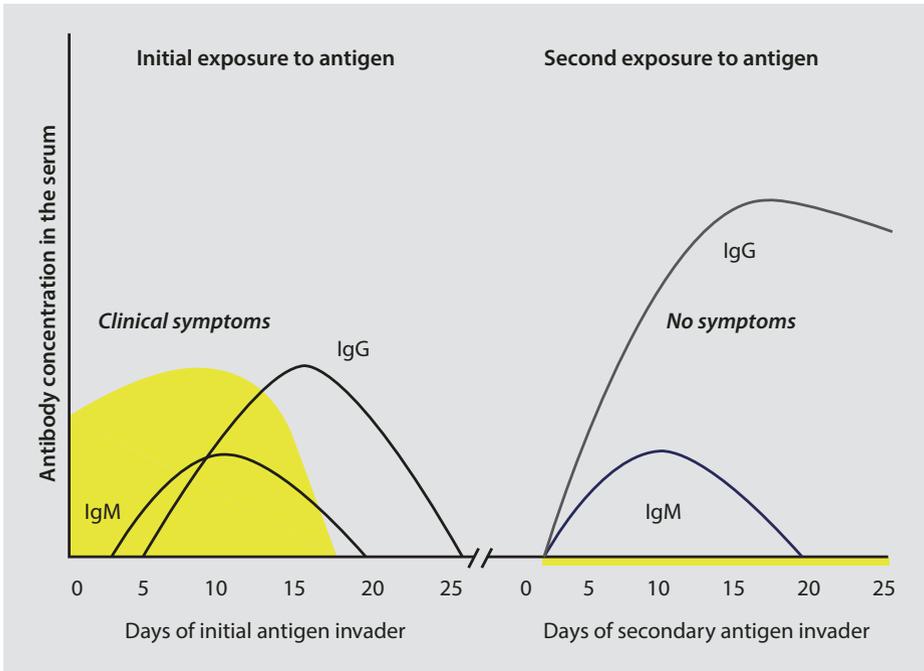
**From a clinical viewpoint,** different mutations such as TNFSF5 on Xq26.3 (type 1), AICDA on 12p13.31 (type 2), CD40 on 20q13.12 (type 3), unknown (type 4), and UNG on 12q24.11 (type 5) lead to inability of B cells to undergo antibody isotype switching, whereas the underdevelopment of secondary lymphoid organs also occurs (*types 1–5 Hyper-IgM syndrome*).

Lymphocytes differentiated during maturation as an increased number of those effector T cells and B cells, which were once exposed to the pathogen, fight it, but being short-lived survive only several days.

### 4.7.1 Memory Cells

However, some lymphocytes are destined to live on as memory T cells, memory B cells, and long-lived plasma cells. These long-lived and even lifelong lymphocytes will protect the body against repeated attacks by the same pathogen. Due to this subset of memory cells, the responses to subsequent infectious attacks are faster and higher than the first response. This explains why once a human has had an infection, he or she does not get this infection when exposed to it the next time around. Prior vaccination also results in the formation of memory cells.

*Memory T cells* are characterized by CD45RO<sup>+</sup> phenotype, rapid HLA-independent turnover, and the ability to secrete much higher levels of cytokines. Recently, memory



■ Fig. 4.6 Increase in IgG due to memory B cells in the secondary infection

T-cell populations were categorized into *central memory T cells* ( $T_{CM}$ ), located in the secondary lymphoid tissues and blood, and *effector memory T cells* ( $T_{EM}$ ), which continuously migrate between peripheral tissues, the blood, and the spleen.

About 40% of B cells in adults are *memory B cells*, and several subsets have been identified. Besides IgG+/IgA+ memory B cells, half of the peripheral blood memory B cells express IgM often without IgD. Reactivated IgG B cells differentiate directly into plasma cells. A common phenotypic marker of memory B cells is  $IgD-IgM + IgG + IgA + CD27+$ . You can see in ■ Fig. 4.6 how IgG concentration increases due to the memory B cells.

#### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

In the course of adaptive immune responses, lymphocytes cannot differentiate into:

1. Inflammatory CD4+ T cells.
2. Cytotoxic CD8+ T cells.
3. Naive lymphocytes.
4. Plasma cells.

**? Question 2**

Cloned daughter lymphocytes can differentiate into:

1. Effector lymphocytes.
2. Naive lymphocytes.
3. Follicular dendritic cells.
4. Macrophages.

**? Question 3**

Cloned daughter lymphocytes can also differentiate into:

1. Naive lymphocytes.
2. Thymocytes.
3. Type 1 dendritic cells.
4. Memory cells.

**? Question 4**

In the course of immune response, maturation of T cells proceeds in:

1. Paracortical zones of the lymph nodes.
2. Follicles of MALT.
3. Secondary follicles of the lymph nodes.
4. The thymus.

**? Question 5**

Differentiation of B cells mainly proceeds in:

1. Paracortical zones of the lymph nodes.
2. Secondary follicles of the lymph nodes and MALT.
3. Periarteriolar lymphatic sheaths of the spleen.
4. Parafollicular zones of the MALT.

**? Question 6**

Differentiation of B cells also proceeds in:

1. Parafollicular zones of the MALT.
2. Periarteriolar lymphatic sheaths of the spleen.
3. The bone marrow.
4. Paracortical zones of the lymph nodes.

**? Question 7**

During maturation, T cells change:

1. Their morphological shape.
2. Their TCRs.
3. Their phenotype.
4. Their coreceptors.

**? Question 8**

During maturation, B cells change:

1. Their morphological shape.
2. Their coreceptors.
3. Their link to simple B-cell-mediated immune response.
4. Their link to advanced B-cell-mediated immune response.

**4** **Question 9**

During maturation, T cells lose the expression of:

1. VLA-4.
2. L-selectins.
3. LFA-1.
4. CD2.

**Question 10**

Differentiation of T cells proceeds faster than B cells:

1. Yes.
2. No.
3. Unknown.
4. Research is in progress.

**Question 11**

Differentiation of B cells proceeds faster than T cells:

1. No way.
2. Research is in progress.
3. Yes.
4. Unknown yet.

**Question 12**

High-affinity B cells are generated in:

1. Plasma cells.
2. Memory T cells.
3. Dendritic cells.
4. Macrophages.

**Question 13**

Memory T cells are characterized by:

1. CD45RA<sup>+</sup> phenotype.
2. IgD-IgM + IgG + IgA + CD27<sup>+</sup> phenotype.
3. CD45R0<sup>+</sup> phenotype.
4. CD16<sup>hi</sup>56<sup>lo</sup> phenotype.

**Question 14**

Memory B cells are characterized by:

1. CD45R0<sup>+</sup> phenotype.
2. IgD-IgM + IgG + IgA + CD27<sup>+</sup> phenotype.
3. CD34<sup>+</sup> phenotype.
4. CD45RA<sup>+</sup> phenotype.

**Question 15**

Memory T cells are divided into:

1. Central memory and effector memory cells.
2. Long-lived plasma cells and memory B cells.
3. Type 1 and type 2 dendritic cells.
4. M1 and M2.

**? Question 16**

Primary exposure to antigen at the initial stage of B-cell maturation leads to:

1. Increase in IgA.
2. Decrease in IgM.
3. Increase in IgD.
4. Increase in IgM.

## 4.8 Effector Activity

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### Definitions

*Immune inflammation* is a type of inflammation, which occurs at the effector stage of adaptive immune responses.

*Immune complex* is the compound of antibody specifically bound to an antigen to neutralize it.

In most cases, T-cell-mediated and B-cell-mediated adaptive immune responses also involve innate immunity and occur at the same time. The effector mechanisms in adaptive immune responses and the spectrum of involved molecules and cells of innate immunity differ between T-cell-mediated and B-cell-mediated responses. In contrast to memory cells, effector cells have a life span of only a few days.

### T-cell-mediated responses:

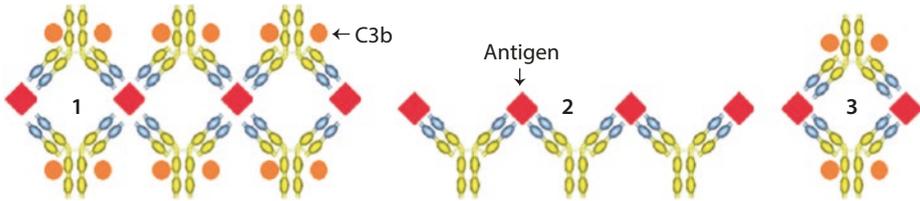
- Apoptosis due to CD8+ T cells and engaged cells and molecules
- Immune inflammation due to CD4+ T cells and involved macrophages and other cells as well as molecules

### B-cell-mediated responses:

- Immune inflammation due to the neutralization of pathogens by antibodies, formation of immune complexes, activation of complement, and phagocytosis
- Antibody-dependent cellular cytotoxicity (ADCC)

In addition, all effector cells and molecules may be categorized as follows: (1) directed against exogenous pathogenic microbes *defensive effectors* that lead to *immune clearance*; (2) directed against reactivated opportunistic microbes of the body deterrent effectors that result in a halt to reactivation, or *immune containment*; and (3) *pathogenic effectors* (e.g., autoantibodies and autoreactive T cells), which may lead to *immunopathology*. The role of so-called witness effectors must be interpreted in each case. For example, antibodies to intracellular pathogens are “witness antibodies.”

Effectors of the T-cell-mediated immune responses destroy cells infected by viruses and other intracellular parasites. These pathogens are eradicated by cytotoxic CD8+ T cells, which engage NK cells and interferons (IFNs) for apoptosis in the target cells, and by inflammatory CD4+ T cells, which activate infected macrophages and a variety of cell types and cytokines to induce *immune inflammation*.



1 - a large immune complex, 2 - a medium-sized immune complex, 3 - a small immune complex

## 4

■ Fig. 4.7 Immune complexes

The effector mechanism performed by inflammatory CD4<sup>+</sup> T cells is not evolutionarily finished because it does not always lead to the effective elimination of the intracellular pathogens. Instead, *from a clinical viewpoint*, macrophages activated by CD4<sup>+</sup> T cells result in long-term *chronic immune inflammation*, the formation of granulomas, angiogenesis, and even destruction of self-tissues. This disorder is linked to *type IV hypersensitivity*.

In the eradication mechanism performed by cytotoxic CD8<sup>+</sup> T cells, molecules triggering cell destruction and cell death include (1) perforin, a protein that perforates the target cell membrane; (2) granzyme, a protease made by perforin and injected into pores; and (3) caspases, which become active through partial degradation by granzyme, constitute caspase cascade, and induce *apoptosis* in the target cell. For CD8<sup>+</sup> T-cell-mediated cytotoxicity, FasL-Fas (CD95) a dependent pathway of apoptosis is not essential. Engaged by some cytokines and activated by IFNs and other cytokines, NK cells exert similar cytotoxicity via apoptosis. Macrophages also contribute directly to this effector mechanism by cytotoxic activity through molecules found on the cell surface or ROS including nitric oxide release from themselves.

Specific antibodies, effector molecules of the B-cell-mediated responses, have several modes of action, which engage cells and molecules of the innate immunity. The antibodies are directed against extracellular microbes.

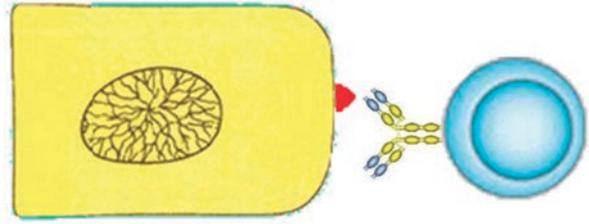
There occurs *neutralization* in which specific antibodies bind antigens and can block parts of the surface of pathogens to inactivate them. During this process, the formation of *immune complexes* involves an antibody, an antigen, and, in some types of immune complexes, complement. If the immune complexes comprise IgG and IgA of high affinity for an antigen, the inactivation of a pathogen will be effective.

The immune complexes may be large, medium-sized, and small (see ■ Fig. 4.7). Large immune complexes contain many molecules of antibodies and complement, i.e., they are formed in excess of antibodies; hence they may be carried by erythrocytes, which express CR1, in the liver and spleen, and then be well-phagocytosed.

The small immune complexes constituted in the slight excess of antigen also comprise complement and can be carried by erythrocytes and phagocytosed too. However, they can be deposited in the tissues. The medium-sized immune complexes, formed in excess of antigen, lattice-like and C3b-free, are insoluble and cannot be lightly removed. *From a clinical viewpoint*, if the immune complexes are not eliminated from the circulation, they are deposited in organs, in particular, vessel walls, and may cause chronic inflammatory diseases based on type III hypersensitivity.

Lysis of the pathogens occurs if, during the binding of an antibody to the antigen, immune complexes fix *complement*, which is *activated* via a classical pathway.

■ **Fig. 4.8** Antibody-dependent cellular cytotoxicity (ADCC)



Antibodies may be opsonins, sticking to pathogens, which results in *phagocytosis* of the pathogens. The formation of immune complexes, activation of complement, opsonization, and phagocytosis finally lead to the development of *immune inflammation*.

In *antibody-dependent cellular cytotoxicity (ADCC)*, Fc $\gamma$ R<sub>s</sub> on the surface of innate effector cells (NK cells, macrophages, and eosinophils) bind to the Fc fragment of a specific antibody, IgG, which itself is bound to a target cell (see ■ Fig. 4.8). ADCC's effector function is potent for human IgG1 and IgG3 and weak for IgG2 and IgG4. According to signaling, a variety of substances such as lytic enzymes, perforin, granzymes, and TNF are released and mediate the destruction of the target cell. This mechanism is available in graft rejection and defense against helminth and protozoan parasites.

#### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

In the course of adaptive immune responses, effector lymphocytes are not:

1. Inflammatory CD4<sup>+</sup> T cells.
2. Cytotoxic CD8<sup>+</sup> T cells.
3. Naive lymphocytes.
4. Plasma cells.

#### ? Question 2

Effector lymphocytes have a life span of:

1. A few days.
2. Many years.
3. About 2 years.
4. Some hours.

#### ? Question 3

Memory T and B cells have a life span of:

1. Some hours.
2. A few days.
3. About 3 years.
4. Many years.

**4** **Question 4**

Effector activity of cytotoxic CD8+ T cells implies:

1. Immune inflammation.
2. Apoptosis in target cells.
3. Neutralization of antigens by antibodies.
4. Complement activation.

**Question 5**

Effector activity of effector CD4+ T cells implies:

1. Apoptosis in target cells.
2. Phagocytosis of pathogens.
3. Neutralization of antigens by antibodies.
4. Immune inflammation.

**Question 6**

Effector activity in the course of B-cell-mediated immune responses implies:

1. "Acute phase" reaction.
2. Apoptosis in target cells.
3. NETosis.
4. Neutralization of antigens by antibodies.

**Question 7**

Plasma cells in the course of simple B-cell-mediated immune response can produce:

1. IgG only.
2. All isotypes of immunoglobulins.
3. IgM only.
4. IgE.

**Question 8**

Plasma cells in the course of advanced B-cell-mediated immune response can produce:

1. IgM only.
2. IgG only.
3. All isotypes of immunoglobulins.
4. No antibodies.

**Question 9**

Pathogenic effectors are:

1. "Witness antibodies."
2. Autoantibodies and autoreactive T cells.
3. Effectors, which are able to fulfill the immune clearance.
4. Effectors, which are able to fulfill the immune containment.

## 4.8 • Effector Activity

**? Question 10**

Deterrent effectors lead to:

1. Immunopathology.
2. A halt of reactivated opportunistic microbes.
3. "Acute phase" reaction.
4. The immune clearance.

**? Question 11**

Large immune complexes are formed in:

1. A slight excess of antigens.
2. Any concentration of antigens.
3. An excess of antibodies.
4. An obvious excess of antigen.

**? Question 12**

Medium-sized immune complexes are constituted in:

1. An obvious excess of antigen.
2. A slight excess of antigens.
3. Any ratio of antigens and antibodies.
4. An excess of antibodies.

**? Question 13**

Effector activity of antibodies cannot involve:

1. Phagocytes.
2. Complement.
3. NK cells.
4. CD8+ T cells.

**? Question 14**

Antibody-dependent cellular cytotoxicity (ADCC) implies the involvement of:

1. Type 1 and type 2 dendritic cells.
2. NK cells, macrophages and eosinophils.
3. Basophils and mast cells.
4. CD4+ T cells.

**? Question 15**

Macrophages can participate in:

1. CD4+ T-cell effector activity only.
2. ADCC only.
3. All types of the effector activity.
4. B-cell-mediated effector activity only.

**Question 16**

Macrophages cannot take part in CD8+ T-cell effector activity:

1. They cannot.
2. Unknown.
3. Research is in progress.
4. They can.

## 4 4.9 Regulation of Immune Responses

### Definitions

*Idiotypic/anti-idiotypic network* is idiotypic-anti-idiotypic interactions between antigen-directed molecules (TCR or BCR), which are called idiotypes, and constituted due to anti-idiotypic responses anti-idiotypes structurally complementary to idiotypes.

*Nonadaptive immunoregulatory cells (Treg and Breg)* are constantly present in the immune system regardless of adaptive immune responses.

*Adaptive immunoregulatory (helper) T cells* are formed in the course of adaptive immune responses.

*Type 1 helper T (Th1) cells, type 2 helper T (Th2) cells, follicular helper T (Tfh) cells, T follicular regulatory (Tfr) cells, type 9 helper T (Th9) cells, type 17 helper T (Th17) cells, and type 22 helper T (Th22) cells* are the different types of adaptive helper T cells.

*Neurotransmitters* are neuromediators, which transmit signals between neurons, muscle cells, endocrine gland cells, and cells of the immune system.

*V genes rearrangement* is a type of rearrangement of B-cell and T-cell V genes to achieve the required specificity of effector molecules at the end of adaptive responses because the initial antigen may not be completely complementary to BCR or TCR of the appropriate lymphocyte clone.

*Somatic hypermutations* are the molecular changes in the V genes of immunoglobulins in the course of B-cell-mediated responses that lead to the increase in antibodies affinity.

*Immune response power* is a type of genetic regulation based on that grooves of different Class I and Class II HLA molecules to upload antigens with a different efficacy.

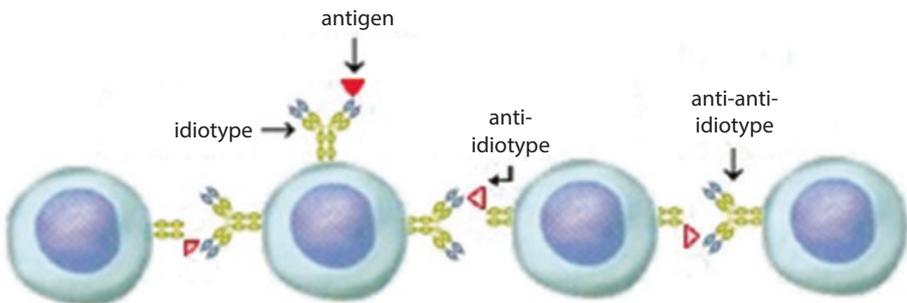
Adaptive immune responses are processes, which are well regulated. The regulation counts for much to achieve the specificity of antibodies and TCRs on effector T cells concerning causative antigen, form the immune memory, restrict the effector activity to protective goals and avoid overactivation of the immune system. Naturally, such events as cytokine storm, autoimmune disorders, and allergic conditions are undesirable. There are at least four levels of regulation:

- Immune system-derived regulatory mechanisms
- Hepatic control
- Neuroendocrine regulation
- Genetic regulation

Immune system-derived mechanisms include the negative feedback model, idiotype/anti-idiotypic network, and regulation by natural immunoregulatory lymphocytes and helper T cells, which exploit cytokines and costimulatory molecules (ergotypes).

### 4.9.1 The Idiotypic/Anti-idiotypic Network

A sample of the feedback principle concerning immune processes is the idiotypic/anti-idiotypic network (see ■ Fig. 4.9). Any antigen-specific molecule (immunoglobulin or TCR) has the unique conformation to its binding site, which is complementary to the shape of antigen. This idiotype may become the focus of the immune response by which the anti-idiotypic molecule is forming. A structure of this molecule would be the “internal image” of the antigen. There are two sorts of anti-idiotypic antibodies: homo-anti-idiotypic (directed against an antigen-binding site) and epi-anti-idiotypic (directed against a part of a Fab fragment). The anti-idiotypic molecules as receptors can in turn either recognize the binding site or their parts themselves or recognize other determinants outside to form a stable network of interacting receptors. When the known antigen is added to the balanced network, the first idiotypic antibody will expand to bind the antigen; the next anti-idiotypic antibody will be produced to bind the idiotype, and so on. Finally, the network is balanced again to end the primary response. To date, this concept established by 1984 Nobel Laureate N.K. Jerne has many proponents and detractors. There is even an idea that the idiotypic/anti-idiotypic network could be exploited to generate idiotypic vaccines.



■ Fig. 4.9 Idiotypic/anti-idiotypic network

## 4.9.2 Natural T-Regulatory Cells

*Natural T-regulatory (nTreg) cells* are a nonadaptive immunoregulatory cell type, which the body employs to control immune homeostasis in the periphery through tolerance to autologous cells. These cells develop in the thymus from positively selected thymocytes and may first be seen during the single-positive stage of T lymphopoiesis. There are several groups of nTreg. Most nTreg cells express  $CD4^+ CD25^{hi}$ , positive for the transcription factor *FoxP3* and represent approximately 5–10% of the total  $CD4^+$  T cell population. They mainly function in three ways: (1) competing with proliferating lymphocytes for IL2 by CD25 molecule, a part of the IL2 receptor; (2) inducing immunosuppressive cytokines such as TGF $\beta$ , IL10, and IL35; and (3) triggering apoptosis in target cells. Interestingly, an increase in the nTreg cells is seen in tumor growth and helminth invasion, whereas a decrease in the cells is found in atopic allergic diseases and autoimmune disorders.

Some nTreg cells, less well characterized, express  $CD8^+ CD25^{hi}FoxP3$ .  $CD8^+$  Treg are able to downregulate  $CD4^+$  Treg in the presence of antiCD3 antibodies in vitro.

Induced nTreg subsets are *iTreg*, *type 3 helper T (Th3) cell*, and *type 1 regulatory T (Tr1) cell*. iTreg cells produce TGF $\beta$  in the placenta that is essential for the normal development of the fetus. Th3 cells secrete TGF $\beta$  and take part in tolerance induction at the mucosal level. Tr1 cells produce IL10 and are also important for the development of mucosal tolerance.

*Natural B-regulatory (nBreg) cells* have recently been discovered. These cells express  $CD19^+ CD24^{hi}CD38^{hi}$  and are probably involved in immune tolerance to autologous cells. nBregs secrete the same immunosuppressive cytokines as nTreg in which they cooperate in the periphery.

## 4.9.3 Adaptive Helper T Cells

*Classical Th1/Th2 paradigm.* About 30 years ago, Coffman and Mossman first divided all helper T cells into two helper subsets, Th1 and Th2 cells. At the basic level, *type 1 helper T (Th1) cells* trigger the T-cell-mediated immune responses to complete effector  $CD4^+$  T cells and cytotoxic  $CD8^+$  T cells. Th1 cells also take part in antibody switching. On the contrary, *type 2 helper T (Th2) cells* upregulate the advanced B-cell-mediated immune response only. Both helper subsets can inhibit each other and cancel opposite effects to reorder the immune response pathway.

Under an immunopathologic Th1 polarization condition, Th1 cells are overactivated in intracellular infections, Th1-type autoimmune diseases, repeated spontaneous abortion, etc. The Th2 polarization condition is present in atopic diseases, Th2-type autoimmune diseases, survival of HLA-restricted fetal allograft, etc.

However, the Th1/Th2 paradigm taken in its simplistic form is prone to a number of paradoxes and exceptions. This model may not be related to processes at the mucosal level during the defense against some intracellular microbes, concerning experimental allergic encephalomyelitis, etc.

Nowadays, some novel immunoregulatory cells including helper T cells and ILC have been discovered (see ■ Table 4.4).

■ **Table 4.4** Some adaptive helper CD4+ T cells

Subset	<i>Th1</i>	<i>Th2</i>	<i>Th17</i>	<i>Th22</i>
Name	Type 1 helper T cell	Type 2 helper T cell	Type 17 helper T cell	Type 22 helper T cell
Phenotype	CD4+	CD4+	CD4+	CD4+
Chemokine receptors	CCR5 CXCR3	CCR3 CCR4	CCR6	CCR10
Cytokines which promote generation	<i>IL12</i> , $IFN\gamma$	<i>IL4</i>	<i>IL23</i> , <i>IL12</i> , <i>IL1</i>	<i>IL6</i> , $TNF\alpha$ , PDGF
Signaling	T-bet	GATA3	ROR $\gamma$ t	AHR
Cytokine profile (key cytokines)	<i>IFN\gamma</i> , <i>IL2</i> , $TNF\beta$ , and <i>IL18</i>	<i>IL4</i> , <i>IL5</i> , <i>IL6</i> , <i>IL10</i> , <i>IL13</i> , and <i>IL33</i>	<i>IL17</i> , <i>IL21</i> , <i>IL22</i> , and <i>CCL20</i>	<i>IL22</i> , <i>IL13</i> , <i>FGF</i> , $TNF\beta$ , <i>CCL15</i> , and <i>CCL17</i>
Target cells	T cells, $\beta$ cells, macrophages, dendritic cells	$\beta$ cells, eosinophils, mast cells	T cells, $\beta$ cells, neutrophils, epitheliocytes	T cells, $\beta$ cells, epitheliocytes, fibroblasts, hepatocytes, neurons
Functional activity	T-cell-mediated and $\beta$ -cell-mediated responses (antibody switching), defense against intracellular pathogens, activation of macrophages	$\beta$ -cell- mediated responses, defense against parasites	Pro- inflammatory effects on mucosae and skin, defense against opportunistic infections	Preferentially anti- inflam- matory effects on mucosae and skin, tissue regeneration
Pathological conditions	Type IV hypersensi- tivity and autoim- mune diseases	Type I hypersensitiv- ity (IgE- dependent diseases)	Autoimmune diseases	Chronic inflammation, autoimmune disorders
Cooperation	Th17, ILC1, and ILC17	Tfh, Th22, Th9, ILC2, and ILC22	Th1, ILC1, ILC17, and phagocytes	Th2, Th9, ILC2, ILC22, and Th17

*Follicular helper T (T<sub>fh</sub>) cells*, a distinct subset of helper CD4+ T lymphocytes, are localized in B-cell follicles of the lymph nodes, spleen, and MALT, where they migrate and generate due to CXCL13 (BLC), *IL6* and *IL21*. Next, upon exposure to foreign antigens, they interact with germinal center B cells and secrete *IL21* to upregulate antibody switching, the hypermutations in B cells, generation of antibody-producing plasma cells, and lifelong memory B cells. At the immunopathologic level, T<sub>fh</sub> cells may take

part in autoimmune disorders. It is possible that Tfh cells may be like a branch in the Th2 differentiation pathway, but their precise lineage relationship to other types of helper CD4<sup>+</sup> T cells is still unclear.

A novel subset, *T follicular regulatory (Tfr) cells*, secretes IL10 and limits the development of germinal center B cells.

*Type 9 helper T (Th9) cells*, a minor helper CD4<sup>+</sup> subset, closely cooperate with Th2 cells and Th22 cells. Activated naive CD4<sup>+</sup> T lymphocytes differentiate into Th9 cells in the presence of IL4 and TGFβ. The Th9 cells themselves secrete IL9 and IL10 to affect mast cells, eosinophils, barrier epitheliocytes, and CD8<sup>+</sup> T cells and promote host defense against parasitic invasion and tumors. Under an immunopathologic Th9 polarization condition, they participate in chronic allergic inflammation and autoimmune disorders.

*Type 17 helper T (Th17) cells* develop from naive CD4<sup>+</sup> cells at the skin and mucosal level where they migrate under the influence of CCL6 and generate due to IL23 and other cytokines. Cytokines produced by Th17 cells can have both physiological and pathogenic effects. The key cytokine is IL17, which upregulates neutrophils, T cells, B cells, and epitheliocytes. The Th17 cells are crucial in host defense against extracellular pathogens located on the mucosae and in the skin and exert synergism to the Th1 cells and some ILC. However, they may take part in such autoimmune processes as rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriasis, type I diabetes mellitus, etc. if an immunopathologic Th17 polarization condition occurs.

Activated naive CD4<sup>+</sup> T cells migrate to the skin and mucosae due to CCL27 (CTACK) and CCL28 (MEC) and differentiate into *type 22 helper T (Th22) cells* in the presence of TNFα and IL6. The Th22 cells affect barrier epitheliocytes, hepatocytes, and neurons through their key cytokine, IL22, and some other cytokines, TNFβ and IL13. They may exert both anti-inflammatory and pro-inflammatory effects since they may function in a synergic manner with both Th2 cells, Th9 cells, some ILC, and even Th17 cells. The physiological effects of Th22 cells are the upregulation of regeneration and host defense against extracellular pathogens on the mucosae and skin. Under an immunopathologic Th22 polarization condition, the Th22 cells may be involved in chronic inflammation related to allergies and autoimmunity.

**From a clinical viewpoint,** the number of *nTreg cells* is increased in lots of types of cancer and parasitic invasions. Conversely, in atopic allergic and autoimmune diseases, the number of *nTreg cells* is diminished. Many types of immunopathology may be divided into some separate groups depending on deviations of various adaptive helper T-cell subsets. However, there are no developed exact diagnostic criteria yet.

#### 4.9.4 Hepatic and Metabolic Control

The liver as the main biochemical “laboratory” provides a common metabolic regulation of homeostasis. The liver is the home to almost all types of the immune system's cellular and subcellular components. The immunoregulatory functions of the liver are as follows:

- Synthesis of most structural components of the immune system
- Oxidation of low-molecular xenobiotics to complete antigens

- Clearance of the immune complexes by Kupffer's cells
- Synthesis of some immunosuppressive factors such as  $\alpha$  fetoprotein (AFP)
- Participation in B lymphopoiesis during fetal life

Some substances and trace elements important for the metabolism are also crucial for both innate and adaptive immunity and immune regulation. There are cholecalciferol (or vitamin D<sub>3</sub>) and other vitamins, squalene, copper, zinc, selenium, etc. Nutritional and endogenous deficiency of these metabolites may lead to acquired immunocompromised condition and diminish outcomes of vaccination. Therefore, some of them are currently manufactured as medications for the immune enhancement therapy and included as adjuvants in vaccines.

### 4.9.5 Neuroendocrine Regulation

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Well-known researcher H. Selye first called attention to neuro-immune interactions. On the one hand, the primary and secondary lymphoid organs are innervated by the nervous system, and, on the other hand, the cells of the immune system express receptors for neurotransmitters and neuropeptides. Through neurotransmission and the hypothalamus-pituitary-adrenal (HPA) axis, any stress including infection affects immune functions.

The nervous and endocrine systems release neurotransmitters, neuropeptides, and endocrine hormones, which modulate a variety of other physiological functions. In turn, the immune system communicates with the nervous and endocrine systems through secreting cytokines and chemokines. Signaling molecules, metabolites, neurotransmitters, and cytokines serve as a communication interface between the central and peripheral nervous systems and immune system, and this communication can work in both directions.

A wide range of pro-inflammatory cytokines such as IL1, IL6, and TNF $\alpha$  can activate the HPA axis and drive increased immune inflammation, but high levels of glucocorticoids lead to the suppression of the immune system and inflammatory processes increasing the value of anti-inflammatory cytokines such as IL10, TGF $\beta$ , and IL35.

Monoamine neurotransmitters, especially norepinephrine, dopamine, and serotonin, are important in regulating the HPA axis and immune system.

*Norepinephrine (noradrenaline)*, a stress-mobilizing sympathetic neurotransmitter, is produced in the brain neurons, especially inside the pons, sympathetic ganglia located near the spinal cord, and adrenal medulla to establish the noradrenergic system. It impacts on the immune system in a modulatory manner. Norepinephrine mainly exerts anti-inflammatory effects by interacting with the adrenoreceptors expressed on lymphocytes and macrophages and inhibiting the production of TNF $\alpha$ , IL1 $\beta$ , and IFN $\gamma$ . However, it may lower the activity of nTreg.

*Dopamine* is a neurotransmitter linked with emotions, the brain's pleasure and reward system. Dopamine is secreted in the brain, kidneys, and cells of the immune system. The direct effects of dopamine on the immune cells are contradictory as they are inhibitory *in vitro*, while in a physiologic concentration, they are mostly stimulatory *in vivo*. During the B-cell-mediated responses, the activity of dopamine in the brain is markedly elevating. It probably shifts the Th1/Th2 balance toward Th1.

*Serotonin* (5-hydroxytryptamine, 5-HT) is a neurotransmitter synthesized by the enterochromaffin cells of GI tract and actively taken up by platelets, basophils, and mast cells. Serotonin transmission between neurons in the brain is responsible for mood, feelings of pleasure, sleep, anxiety, and appetite. Serotonin through the serotonergic system inhibits the production of pro-inflammatory cytokines such as TNF $\alpha$  and IL12 and cancels the Th1 polarization conditions in immunopathology. During B-cell-mediated responses, the activity of serotonin in the brain markedly decreases.

*Acetylcholine*, a parasympathetic neurotransmitter, is produced in the motor neurons, parasympathetic nervous system, and brain to constitute the cholinergic system. It appears to play an immune-inhibitory role in the brain. For example, during B-cell-mediated responses, the activity of acetylcholine in the brain is strikingly lowered, but if inflammation occurs, acetylcholine increases in order to block the process.

$\gamma$ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS). GABA is produced in the GABAergic neurons of the brain and spinal cord. To date, GABAergic mechanisms have been demonstrated throughout the body. As for the immune system, GABA exhibits anti-inflammatory and immunosuppressive effects inhibiting the production of IL1 $\beta$ , IL6, IFN $\gamma$ , and IL17. However, the amount of GABA in the brain during an inflammatory process in the body is increased.

The pituitary gland or hypophysis consists of three lobes: anterior, intermediate, and posterior. The anterior lobe of the pituitary gland synthesizes *somatotropin* (growth hormone), *thyroid-stimulating hormone* (TSH), *adrenocorticotrophic hormone* (ACTH), *lactotropic hormone* (LTH) or *prolactin*,  $\beta$  *endorphin*, and *gonadotropins*. These hormones are released under the influence of hypothalamic releasing factors. The hormones of the anterior lobe exert largely immunostimulatory effects. In particular, somatotropin upregulates T lymphopoiesis in the thymus. However,  $\beta$  endorphin may exhibit immunosuppressive action at a high concentration.

The intermediate lobe produces *melanocyte-stimulating hormone* (MSH), which displays anti-inflammatory and immunosuppressive effects.

The posterior lobe of the pituitary gland is an extension of the hypothalamus, a part of the brain. It produces the *antidiuretic hormone* (ADH), or *vasopressin*, and *oxytocin*. These hormones suppress the HPA axis and exert a mild immunomodulatory action.

*Melatonin*, the pineal gland (or epiphysis) hormone, proved to be a focus of research as a potential factor, which regulates many of the immune processes taken as stress reactions. Regarding the immune system, melatonin, a biorhythmic regulator, exerts an immunostimulatory action as well as a soporific effect, antioxidant activity, and the ability to decrease the cholesterol concentration in the blood.

The thyroid gland produces *triiodothyronine* (T3) and *thyroxine* (T4) that impact on the immune system mainly in an upregulating manner. Unfortunately, there are some autoimmune thyroid diseases caused by a breakdown in immune tolerance under the influence of a wide range of harmful factors.

The pancreas is an endocrine gland producing several important hormones such as *insulin* ( $\beta$  cells), *glucagon* ( $\alpha$  cells), and *somatostatin* ( $\delta$  cells), which have immunomodulatory and mainly upregulatory effects. Type 1 diabetes mellitus is a severe autoimmune disease due to the autoimmune damage to the pancreatic structure. Furthermore, insulin is a powerful mitogen, which may be taken into consideration as a factor of the carcinogenesis if the insulin resistance and hyperinsulinemia occur.

The adrenal gland produces over 30 different hormones including steroids in the cortex and norepinephrine and epinephrine in the medulla. The zona glomerulosa of the cortex secretes mineralocorticoids including aldosterone, the zona fasciculate produces glucocorticoids, and the zona reticularis secretes dehydroepiandrosterone (DHEA), androgen, and estrogens.

*Aldosterone* as a part of renin-angiotensin-aldosterone system (RAAS) is critically involved in the pathogenesis of hypertension. It has been shown that RAAS may play a pathological role in the immune mechanisms associated with hypertension.

*Glucocorticoids* exhibit their immunosuppressive effects due to inhibition of cytokine expression at the transcriptional level.

The most abundant adrenal steroid, DHEA, which lacks sexual activity, is a keystone intermediate in the metabolic pathways of sex hormones. It has been demonstrated in research of the interactions of estrogen and androgen receptors on lymphocytes that *estrogens* promote Th2 polarization conditions and inhibit a Th1 polarization condition, whereas *DHEA* and *androgens* have the reverse action.

**From a clinical viewpoint,** the nervous system and endocrine glands sometimes undergo autoimmune attacks that lead to the development of such diseases as *multiple sclerosis* and *type I diabetes mellitus*.

### 4.9.6 Genetic Regulation

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During immune responses, the required *specificity* is achieved by *immunoglobulin/TCR gene rearrangement* to obtain a diversity of effector molecules. The gene products of recombination-activating genes (RAG-1 and RAG-2) are particularly required at all stages of recombination for both antibodies and TCR.

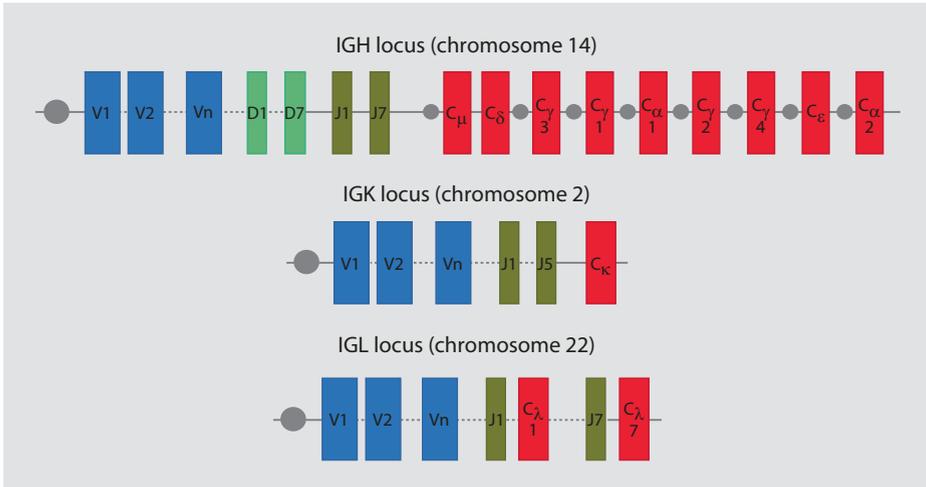
The *immune response power* is provided by Class II HLA and Class I HLA gene products and expression of high powerful cytokines.

### 4.9.7 Generation of Effector Molecule Diversity

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The 1987 Nobel Laureate Susumu Tonegawa elucidated the genetic principle of the adaptive immunity, in particular, the genetic mechanisms of antibody diversity. Each chain (H and L) of immunoglobulin is encoded by a gene complex. Within it, groups of genes (or segments) are available (see ■ Fig. 4.10). Numerous V genes encode the variable domains, whereas a limited number of C genes encode the constant domains of each chain. There are also other genes responsible for joining these chains (J genes) and provision H chains with an additional antibody diversity (D genes). The main clusters of the immunoglobulin genes are located on different chromosomes:  $\kappa$  genes on chromosome 2 (locus IGK),  $\lambda$  genes on chromosome 22 (locus IGL), and H genes on chromosome 14 (locus IGH).

After antigenic stimulation and priming, the primed B cell DNA is composed of a final gene repertoire to be transcribed onto mRNA and translated onto a peptide chain. During isotype switching, IgM's H chain must be replaced by a selection of the  $C\gamma$ ,  $C\alpha$ , or  $C\epsilon$  gene, which is achieved by the use of switch regions (see ■ Fig. 4.11).



■ Fig. 4.10 Genes of immunoglobulins

*Antibody diversity* allows the immune system to structurally specify the antigen-binding sites of an antibody to a causative antigen. During an advanced B-cell-mediated immune response, antibody diversity is generated by the following mechanisms:

1. A large number of *V* genes are achieved in the course of B lymphopoiesis.
2. *Combinatorial association* may be explained by different combinations of H chains and L chains within a single B cell.
3. *Junctional diversity* is linked to a random manner of joining the VJ and VDJ regions. In addition, during the joining of the VJ and VDJ regions, inaccuracies may take place.
4. *N region insertion* between the VJ and VDJ regions occurs when a series of nucleotides is catalyzed by the enzyme terminal transferase that leads to forming a new hypervariable region.
5. *Somatic hypermutation* occurs only in the *V* genes of B cells. Somatic hypermutation results in approximately one nucleotide change per *V* gene, per cell division.

Analogous to immunoglobulins, TCRs have distinctive segments for the receptor chains:  $\alpha$ - $\delta$  locus (TRA-TRD) on chromosome 14 and  $\beta$  locus (TRB) and  $\gamma$  locus (TRG) on chromosome 7 (see ■ Fig. 4.12). The mechanisms for bringing different gene components of TCRs together appear to be the same as that used for the immunoglobulin genes. However, TCR genes do not mutate somatically after the gene rearrangement, and TCR gene products may function only as membrane-bound molecules. The TCR genes also have wide diversity.

By means of *combinatorial diversity*, the immunoglobulin gene repertoire may be above 50 million. According to *junctional diversity* (in possible frameshift mutations during the joining of two genes), this number may reach  $10^{11}$ . The diversity of TCR genes is also numerous (see ■ Table 4.5).

The *HLA genes* are located within the 6p21.3 region on the short arm of human chromosome 6 and contain more than 220 genes of diverse function.  $\beta_2$  microglobulin

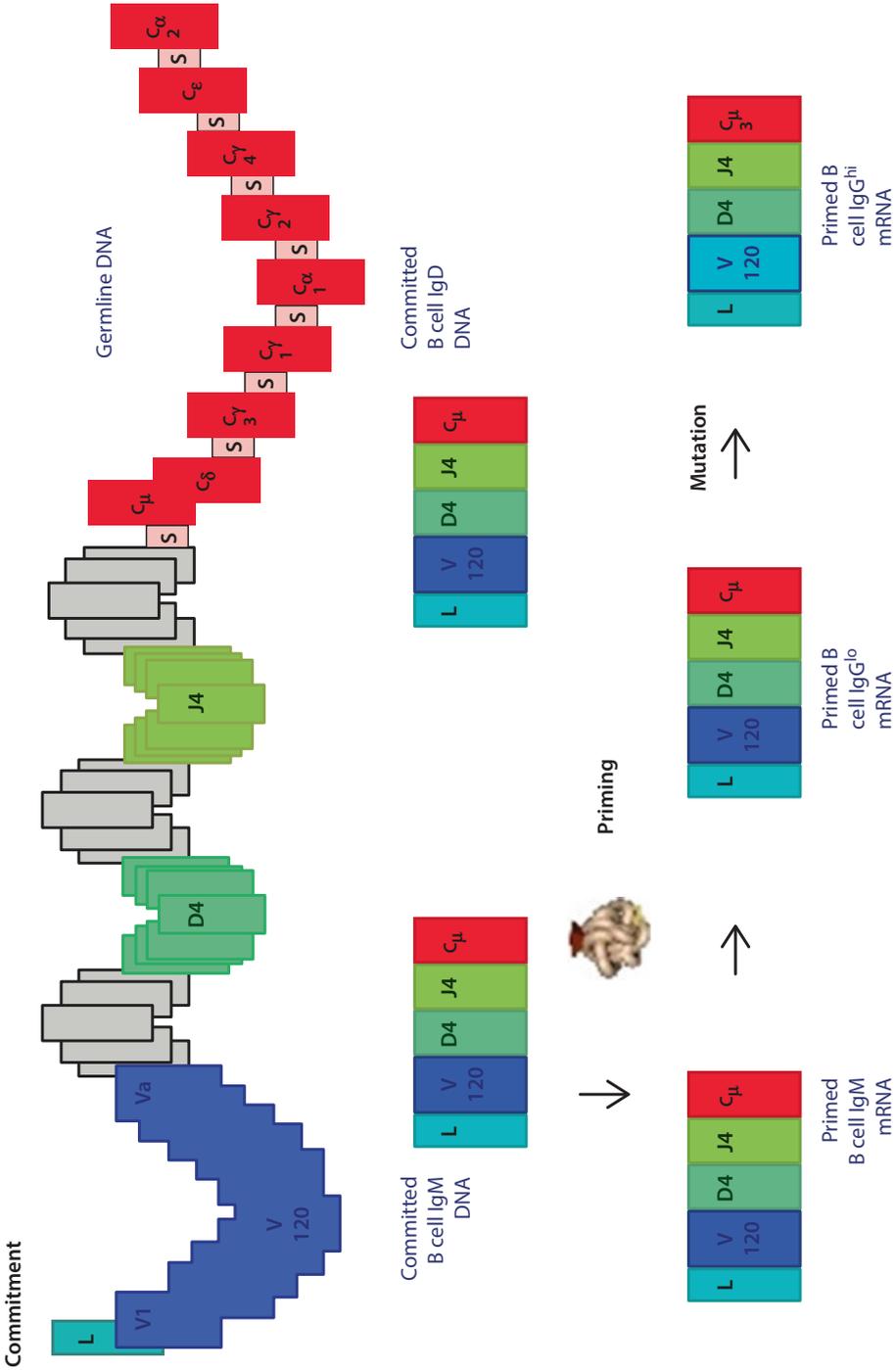
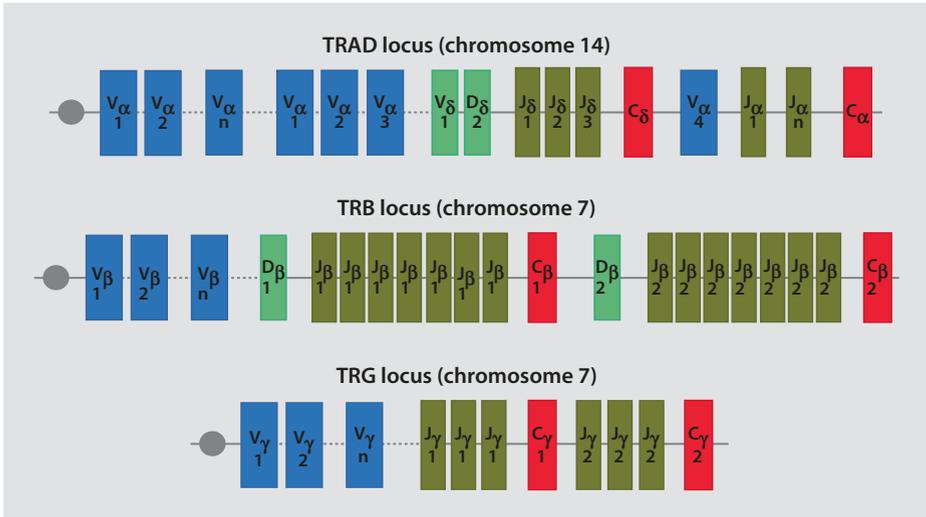


Fig. 4.11 Immunoglobulin gene rearrangement during B-cell-mediated response



■ Fig. 4.12 Genes of TCR

(B2B) is encoded by a gene on chromosome 15. In total, the inheritance of the HLA genes is codominant and follows a Mendelian inheritance pattern.

Among Class I HLA genes, there are three major loci, HLA-A, HLA-B and HLA-C, and three minor loci, HLA-E, HLA-F, and HLA-G. Among Class II HLA genes, there are three major loci, HLA-DP, HLA-DQ, and HLA-DR, and two minor loci, HLA-DM and HLA-DO (see ■ Fig. 4.13).

Each individual has a “full house,” i.e., maternal and paternal haplotypes, which may be determined by HLA typing. It is especially essential during preparation for transplantation of any organ. HLA genes are highly polymorphic and have many different alleles. In the new nomenclature, an allele is first identified by the locus letters (e.g., HLA-A); next an asterisk (\*) follows; then a two-digit number defining the allele group goes; then after a colon (:), there is a three-digit number identifying the specific HLA protein; then after another colon (:), there is a two-digit number defining synonymous DNA substitution within the coding region; and then after a third colon (:), there follows a two-digit number defining the differences in a noncoding region plus a suffix to denote changes in the expression. The suffix may have some meaning: N, “null” alleles; L, low expression; S, secreted but not expressed molecule; C, cytoplasmic presence; A, aberrant expression; and Q, questionable.

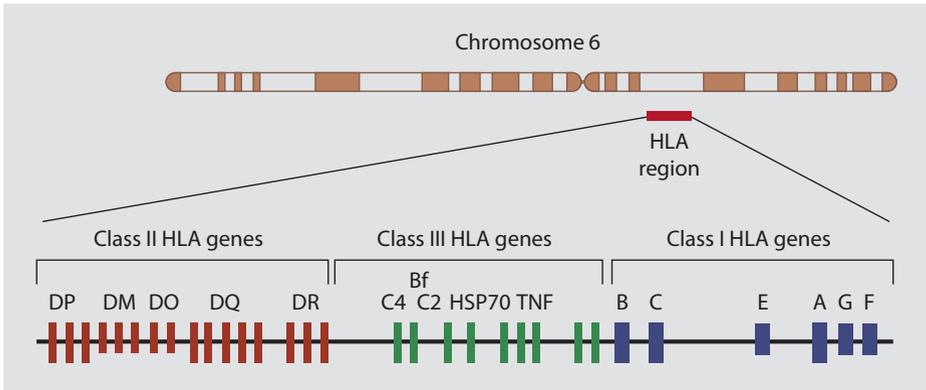
Here you can see an example of an HLA allele designation:

HLA-A\*02:050:01:01C

**From a clinical viewpoint,** the relationship between *HLA genes* and many diseases has not yet been fully studied. However, it is known 90% of people with *ankylosing spondylitis* are HLA-B27 positive.

**Table 4.5** Combinatorial and junctional diversity of immunoglobulin and TCR genes

Criterion	Immunoglobulin genes				TCR genes			
	H	κ	λ		α	β	γ	δ
Main cluster location	14	2	22		14	7	7	14
Orphon location	9, 15, 16	1, 2, 22	–		–	–	–	–
Number of V genes and subgroups	Above 250	Above 250	Above 100		Above 100	Above 25	Above 7	Above 10
Number of D genes	27	–	–		–	2	–	3
Number of J genes	6	5	4		61	13	5	4
Number of C genes	9	1	4		1	2	2	1
Combinatorial diversity	Above 50·10 <sup>6</sup>				About 50·10 <sup>6</sup>			
Junctional diversity	About 3·10 <sup>7</sup>				About 2·10 <sup>11</sup>			
Total diversity	About 10 <sup>14</sup>				About 10 <sup>18</sup>			



■ Fig. 4.13 Genes of HLA molecules

### 4.9.8 Generation of the Power of Immune Responses

Many genes encode the molecules of the immune system. Class I HLA gene products constitute a functional receptor on most nucleated cells of the body and are responsible for superficial polymorphism in humans. In addition, the immune system uses the HLA molecules to differentiate self-cells and non-self cells. If a cell is infected by a virus, the Class I HLA molecules bring fragments of the virus to the surface of the cell so that the cell can be destroyed by cytotoxic CD8<sup>+</sup> T cells. Also, any infected cell loses Class I HLA molecules expressed on the surface of the cell, and it is rapidly attacked by NK cells, triggering apoptosis in it.

Class I and Class II HLA molecules take part in uploading antigenic epitopes on the groove of HLA molecules for presentation to lymphocytes. The groove of a single HLA molecule can accommodate a lot of different antigenic epitopes but cannot bind all epitopes in such a manner in order to reach a high concentration of antibodies and effector T cells. Each individual is able to mount powerful immune responses only to certain antigens and weaker responses to others. That is why HLA genes may be defined as genes whose products control the power of adaptive immune responses.

#### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

Natural Treg are:

1. Adaptive immunoregulatory T cells are:
2. Type 1 helper T cells.
3. Type 17 helper T cells.
4. Nonadaptive immunoregulatory T cells.

**? Question 2**

Type 2 helper T cells secrete:

1. IL4 and IL13.
2. IFN $\gamma$  and IL2.
3. IL21.
4. IL22 and CCL15.

**? Question 3**

Type 1 helper T cells are related to:

1. Adaptive immunoregulatory T cells.
2. Nonadaptive immunoregulatory B cells.
3. Type 17 helper T cells.
4. Nonadaptive immunoregulatory T cells.

**? Question 4**

Follicular helper T cells secrete:

1. IL4 and IL13.
2. IFN $\gamma$  and IL2.
3. IL21.
4. IL17 and CCL20.

**? Question 5**

Follicular helper T cells upregulate:

1. CD8+ -cell-mediated immune response.
2. Phagocytosis.
3. B-cell-mediated immune responses.
4. CD4+ T-cell-mediated immune response.

**? Question 6**

Type 17 helper T cells secrete:

1. IL17 and CCL20.
2. IFN $\gamma$  and IL2.
3. IL4 and IL13.
4. CCL15 and CCL17.

**? Question 7**

Type 2 helper T cells upregulate:

1. CD8+ T-cell-mediated immune response.
2. Simple B-cell-mediated immune response.
3. Advanced B-cell-mediated immune response.
4. CD4+ T-cell-mediated immune response.

**? Question 8**

T follicular regulatory (Tfr) cells downregulate:

1. B-cell clonal expansion.
2. CD4+ T-cell clonal expansion.
3. Unknown yet.
4. CD8+ T-cell clonal expansion.

**? Question 9**

Type 22 helper T cells exhibit:

1. Pro-inflammatory effects on mucosae and skin only.
2. Defense against parasites.
3. Preferentially anti-inflammatory effects on mucosae and skin.
4. Effect of macrophage activation.

**? Question 10**

Type 1 helper T cells cooperate with:

1. Type 2 helper T cells.
2. Type 17 helper T cells.
3. Type 22 helper T cells.
4. ILC2.

**? Question 11**

Phenotype of most nTreg is characterized by:

1. CD16<sup>hi</sup>CD56<sup>lo</sup>.
2. CD34+.
3. CD4+ CD25<sup>hi</sup>FoxP3.
4. CD16<sup>lo</sup>CD56<sup>hi</sup>.

**? Question 12**

Classical immunoregulatory paradigm is:

1. Th17/Th22 paradigm.
2. Pattern recognition theory.
3. Th1/Th2 paradigm.
4. Concept "missing self."

**? Question 13**

Glucocorticoids exert:

1. Immunostimulatory effects.
2. Immunosuppressive effects.
3. Unknown.
4. Neither immunostimulatory nor immunosuppressive effects.

**? Question 14**

Estrogens promote:

1. Th1 deviation.
2. Th2 deviation.
3. CD4+ T-cell-mediated immune response.
4. CD8+ T-cell-mediated immune response.

**? Question 15**

A tool for the high-affinity BCR selection is:

1. Antigen processing.
2. "Dual recognition."
3. Somatic hypermutations.
4. Endocytosis of antigens.

**? Question 16**

Enzymes required for this gene rearrangement are:

1. Products of RAG-1 and RAG-2.
2. Phospholipases C $\gamma$ 1 and C $\gamma$ 2.
3. Tyrosine kinases.
4. MX protein GTPases.

## 4.10 Immune Tolerance

---

### Definitions

*Immune tolerance* is the unresponsiveness of the immune system to an antigen (tolerogen).

*Clonal deletion* (the central mechanism) and some peripheral mechanisms including (1) *activation-induced apoptosis*, (2) *clonal anergy*, (3) *clonal ignorance*, and (4) activity of *nonadaptive regulatory lymphocytes* are the physiological mechanisms of tolerance maintenance.

*Immune tolerance*, the opposite of an adaptive immune response, is a state of the immune system when there is unresponsiveness to a specific antigen, which must be called tolerogen. Immune tolerance may be *natural tolerance* or an *artificial state* achieved for medical purposes (e.g., in transplantation). There is also *pathologic tolerance*, which occurs under conditions that pathologically suppress the immune responses to antigens (e.g., in tumor growth and chronic infection).

In early experiments by 1960 Nobel Laureate Sir P.B. Medawar and other researchers, the artificial immune tolerance was achieved by several methods:

- Prior contact with a particular antigen in fetal life or in the newborn period when the immune system is not yet mature
- Prior contact with the antigen in extremely high or low doses
- Exposure to radiation, the introduction of chemotherapy agents, or other substances, which could damage to the immune system

Later, the other 1960 Nobel Laureate Sir F. M. Burnet demonstrated the model of "clonal deletion" as a method by which immune tolerance might be achieved.

Tolerance is commonly accepted to be an active process both at the central and peripheral levels and with both engagements of tolerogenic T cells and B cells. The tolerant state is not absolute and rarely complete. There may be T-cell tolerance, more often low-dose dependent and long term, and B-cell tolerance, more often high-dose dependent and short term. The continuous persistence of tolerogen and its accessibility to the

immune system are certainly required to maintain tolerance, which may be subsequently canceled by newly emerging T cells and B cells.

Under natural conditions, the immune system is tolerant to:

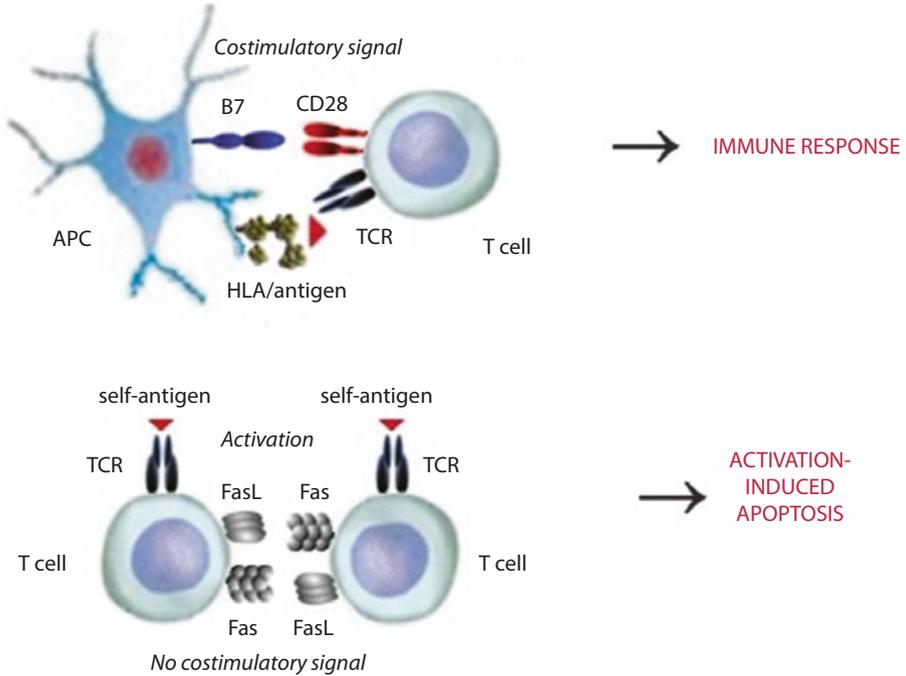
1. Self-antigens (or endo-antigens of the body)
2. Antigens of own symbiotic and opportunistic microbes in the steady state
3. Food proteins
4. Allergens of the environment
5. Antigens of spermatozoa (in women) and father's antigens of the fetus (in pregnant women)

There are a number of physiological processes for the maintenance of natural tolerance:

- Central mechanisms (clonal deletion)
- Peripheral mechanisms:
  - Activation-induced apoptosis
  - Clonal anergy
  - Clonal ignorance
  - nTreg, nBreg, and pro-tolerogenic cytokines

*Central tolerance* of T cells occurs during thymic development with the bulk of self-reactive T cells eliminated during negative selection in the thymus termed *clonal deletion*. However, the thymic selection is not a perfect process, and hence a part of self-reactive T cells may frequently be released into the periphery. Most of the survived self-reactive T cells are low affinity but potentially dangerous for the body as they can be effectively engaged in an autoimmune response. In addition, it is unlikely that all peripheral self-antigens are expressed within the thymus, so high-affinity self-reactive T cells can also enter the bloodstream.

*Activation-induced apoptosis* (see ■ Fig. 4.14) is the apoptosis in self-reactive T cells previously activated through TCR. TCR ligation upregulates CD95 expression and induces the expression of CD95L (CD178). Apoptosis is mediated by the activation of the Fas (CD95) pathway, which is activated upon the ligation of cell surface CD95 by CD95L (FasL) and can occur by the interaction of CD95 and CD95L on the same self-reactive T cell. However, costimulation of T cells by the direct ligation of CD28 inhibits activation-induced apoptosis in these cells. Thus, for activation-induced apoptosis in previously activated self-reactive T cells, maintenance of the natural tolerance, and prevention of autoimmune disorders in target organs, the absence of a costimulatory signal is necessary.



■ Fig. 4.14 Activation-induced apoptosis

*Clonal anergy* is a silent state of self-reactive B cells and T cells in the periphery. The antigen receptors of these cells at the immature stages were directed to soluble self-antigens at a low concentration. That is why these cells escaped from the negative selection in the bone marrow and thymus. In the periphery, these cells have a low expression or/and shedding of antigen receptors, coreceptors, and costimulatory molecules. Respectively, CD28 is decreased in T cells and increased in B cells, and CTLA-4 is increased in T cells and decreased in B cells.

*Clonal ignorance* is the unresponsiveness of T cells in the periphery to self-antigens at a low concentration. However, self-ignorant T cells represent one of the most significant threats to the maintenance of tolerance. There are *immune-privileged organs* such as some parts of the brain, the anterior chamber of the eye, the testes, etc. Anatomical barriers separate the blood from these self-antigens (so-called sequestered or cryptic determinants), but upon certain conditions (e.g., trauma), self-antigens can enter the bloodstream at a high concentration and lead to a tolerance breakdown.

*Natural T-regulatory (nTreg) cells* and *B-regulatory (nBreg) cells* have been described in detail in this chapter. They operate under pro-tolerogenic and immunosuppressive cytokines such as IL10, IL35, and TGF $\beta$ .

*From a clinical viewpoint*, the breakdown of natural tolerance leads to autoimmune diseases, *dysbioses of barrier microbiome*, *food allergies*, *atopic allergic diseases*, and *immunological infertility*.

### 4.10.1 Oral Tolerance

---

Oral administration of an antigen may induce self-tolerance. An antigen in small doses administered orally or intranasally may induce antigen-specific antibodies and increase levels of nTreg and cytokines IL4 and IL10 in gut-associated lymphoid tissue (GALT). An antigen in high dosages may result in the deletion of self-reactive T cells but not promote the generation of nTreg. The precise mechanisms of how an oral antigen induces tolerance are still unclear. It is known that nonadaptive immunoregulatory lymphocyte subsets (nTreg and nBreg) and their induced subpopulations (iTreg, Th3, and Tr1), as well as innate lymphoid cells (ILC), play roles in the induction of oral tolerance.

**From a clinical point of view,** oral tolerance may take into consideration as a possible instrument, which may be useful for a variety of clinical approaches such as sublingual immunotherapy in atopic allergic diseases, preparation for graft transplantation, treatment for food allergies, and autoimmune diseases.

#### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

Immune tolerance is:

1. Immunodeficiency.
2. A form of allergy.
3. Unresponsiveness to a specific antigen.
4. Adaptive immune response to a specific antigen.

#### ? Question 2

Artificial tolerance is required in:

1. Transplantation.
2. Acute infection.
3. Chronic infection.
4. Tumor growth.

#### ? Question 3

This mechanism is not related to the peripheral tolerance:

1. Clonal ignorance.
2. Functioning nTreg.
3. Activation-induced apoptosis.
4. Clonal deletion.

#### ? Question 4

This mechanism is related to the central tolerance:

1. Activation-induced apoptosis.
2. Functioning nTreg.
3. Clonal deletion.
4. Clonal ignorance.

**? Question 5**

These researchers were awarded Nobel Prize for works in the field of immune tolerance:

1. F.M. Burnet and P.B. Medawar.
2. E.E. Metchnikoff and P. Ehrlich.
3. B.A. Beutler, J.A. Hoffmann and R.M. Steinman.
4. P.C. Doherty and R.M. Zinkernagel.

**? Question 6**

For activation-induced apoptosis, a mechanism of immune tolerance is required:

1. The presence of costimulatory signal.
2. The absence of CD95 expression.
3. The absence of costimulatory signal.
4. The absence of CD95L expression.

**? Question 7**

Maintenance of the natural tolerance leads to:

1. Autoimmune disorders.
2. Immunological infertility.
3. Tissue homeostasis.
4. Atopic allergic diseases.

**? Question 8**

Breakdown of the natural tolerance results in:

1. Autoinflammatory disorders.
2. Tissue homeostasis.
3. Physiological pregnancy.
4. Autoimmune diseases.

**? Question 9**

B-cell clonal anergy is characterized by:

1. Increase in CD28 expression.
2. Cross-linking antigens.
3. Clonal deletion.
4. Increase in CTLA-4 expression.

**? Question 10**

T-cell clonal anergy is characterized by:

1. Clonal deletion.
2. Increase in CD28 expression.
3. Increase in CTLA-4 expression.
4. Complement activation.

**4** **?** **Question 11**

Clonal ignorance is:

1. Adaptive immune responses to self-antigens at a low concentration.
2. The activation of innate immunity.
3. The unresponsiveness of peripheral lymphocytes to self-antigens at a low concentration.
4. Adaptive immune responses to self-antigens at a high concentration.

**?** **Question 12**

Natural Tregs secrete:

1. IL12.
2. IFN $\gamma$ .
3. IL4.
4. IL35.

**?** **Question 13**

This subset refers to induced subpopulations of nTregs:

1. Type 1 helper T cell.
2. Type 2 helper T cell.
3. Type 3 helper T cell.
4. Type 9 helper T cell.

**?** **Question 14**

This subset also refers to induced subpopulations of nTregs:

1. T follicular regulatory T cell.
2. Type 1 regulatory T cell.
3. Type 17 helper T cell.
4. Type 22 helper T cell.

**?** **Question 15**

Oral administration of an antigen may induce self-tolerance:

1. Yes.
2. No way.
3. Research has never been carried out.
4. Unknown.

**?** **Question 16**

Immune tolerance may be a medical purpose:

1. Research has never been carried out.
2. No.
3. Unknown.
4. Yes.

### Key Points

1. Adaptive B-cell-mediated responses proceed either as the simple response when the memory to the antigen is not constituting or as advanced B-cell response accompanying the production of all isotypes of antibodies and long-term memory to the causative antigen. Adaptive T-cell-mediated responses may be either CD4+ T-cell or CD8+ T-cell process and in all cases lead to long-term memory to the antigen. B-cell-mediated responses are directed against extracellular antigens, whereas T-cell-mediated responses protect against intracellular antigens.
2. Correspondingly, effector molecules and cells at the effector stage of adaptive immune responses are (1) immunoglobulins, which can neutralize antigens forming immune complexes; (2) immunoglobulins taking part in the antibody-dependent cellular cytotoxicity (ADCC); (3) CD4+ T cells, which induce the immune inflammation; and (4) CD8+ T cells that destroy virus-infected cells and other cell objects via apoptosis.
3. Adaptive immune responses are well-regulated processes. There are immune system-derived mechanisms, hepatic control, neuroendocrine regulation, and genetic regulation. Above all, nonadaptive regulatory lymphocytes and helper T cells count for much in the immune regulation, and classical Th1/Th2 paradigm has currently been updating.
4. Immune tolerance is the unresponsiveness of the immune system to self-antigens, antigens of own symbiotic and opportunistic microbes, food proteins, environmental allergens, and antigens of spermatozoa (in women) and father's antigens of the fetus (in pregnant women). The maintenance of immune tolerance is the important factor for health and well-being.

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# Immunological and Molecular Biological Methods

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**Electronic supplementary material** The online version of this chapter ([https://doi.org/10.1007/978-3-030-03323-1\\_5](https://doi.org/10.1007/978-3-030-03323-1_5)) contains supplementary material, which is available to authorized users.

## Learning Objectives

*Knowledge.* Upon successful completion of the chapter, students should be able to:

1. Describe the principle and known protocols of the flow cytometry and immunohistochemistry. Evaluate the value of this approach for advances in immunology and sister fields.
2. Identify the basic principles of enzyme-linked immunosorbent assay (ELISA).
3. Outline the immunoblot (Western blot).
4. Be familiar with the radioimmunoassay (RIA).
5. Describe the cell proliferation and cytotoxicity assays.
6. Appreciate the tests on phagocytosis.
7. Describe the molecular biological methods and identify the basic principles underlying their protocols.

*Acquired Skills.* Upon successful completion of the chapter, students should demonstrate the following skills, including:

1. Interpret the knowledge related to immunological and molecular biological techniques.
2. Critically evaluate the scientific literature about new methods of immunoassays.
3. Discuss the scientific articles from the current research literature concerning principles and protocols of immunoassays.
4. Attain a clear perception of the presented immunology definitions expressed orally and in written form.
5. Formulate the presented immunology terms.
6. Design immunological experiments to test a certain hypothesis.
7. Analyze data of immunoassays from a clinical viewpoint.
8. Correctly answer quiz questions.

*Attitude and Professional Behaviors.* Students should be able to:

1. Have the readiness to be hardworking.
2. Behave professionally at all times.
3. Recognize the importance of studying and demonstrate a commitment.

## 5.1 Introduction

---

The reader can find the brief description of modern immunological and molecular biological techniques, principles of their execution, clinical applications, and rules of results evaluation in the individual context.

Current immunodiagnosics includes innovative immunoassays and automated immunoanalyzer technologies used for verified clinical diagnostics in patients. Any test should have (1) very high *sensitivity*, i.e., the ability to correctly identify persons who have a disease, and (2) *specificity*, i.e., the ability to correctly identify individuals who do not have a disease. If a test is highly sensitive, it will identify most persons with the disease – in short it will lead to very few false-negative results. If a test is highly specific, only a small number of people will test positive for the disease who do not have it – i.e., it will result in very few false-positive parameters.

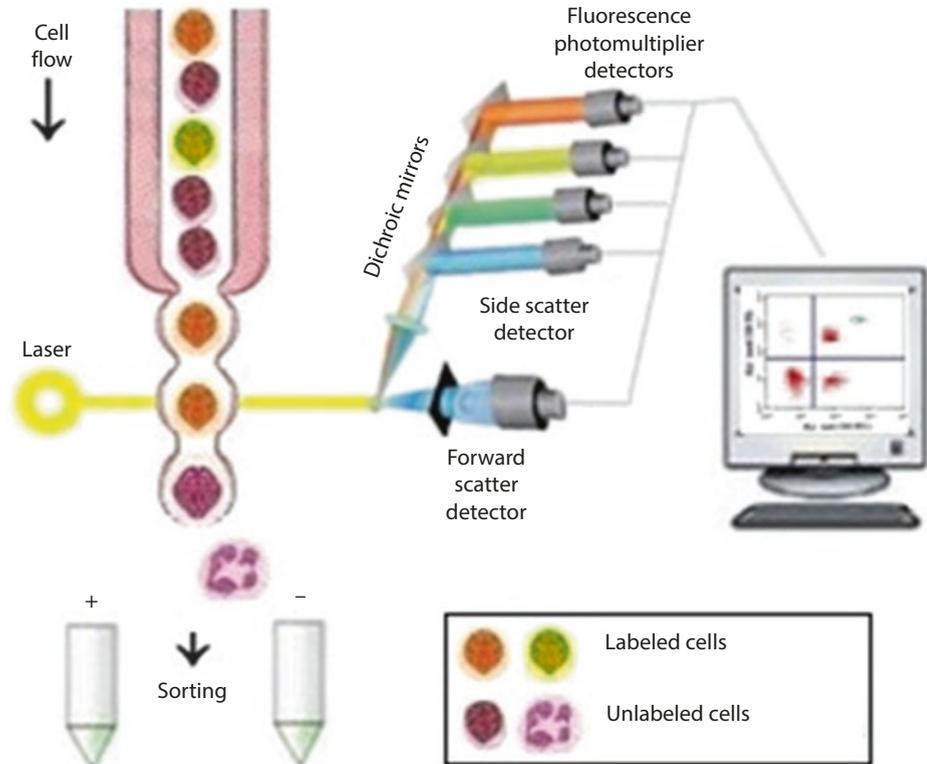
## 5.2 Flow Cytofluorometry

### Definitions

*Flow cytofluorometry* is the technique based on the flow of cells through three types of laser light that allows to analyze a lot of the cell parameters with a great speed.

*Fluorochromes (fluoropores)* are fluorescent dyes, which are used for the visualization in the course of many techniques including the flow cytofluorometry.

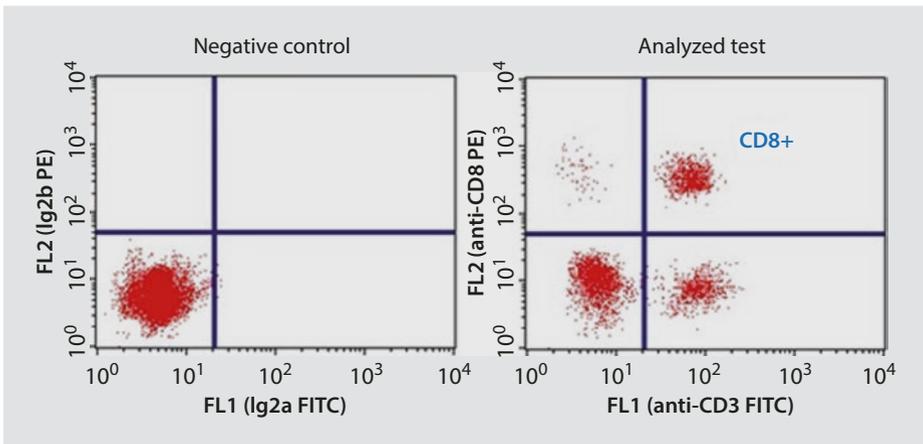
Flow cytofluorometry (FCFM), a method of measuring and analyzing light emissions that interact with particles flowing in a liquid stream, has an enormous number of applications in life science. The technique is based on using three types of laser light: (i) forward scattered light, (ii) orthogonal scattered at  $90^\circ$  light, and (iii) dye-specific fluorescence light (see ■ Fig. 5.1). Cells pass through laser light by a single cell in the flow of a droplet. The light scatter is commonly exploited to exclude dead cells, cell aggregates, and cell debris from fluorescence data. It is also used to assess any granularity and cell size and distinguish lymphocytes, monocytes, and granulocytes from each other in blood leukocyte



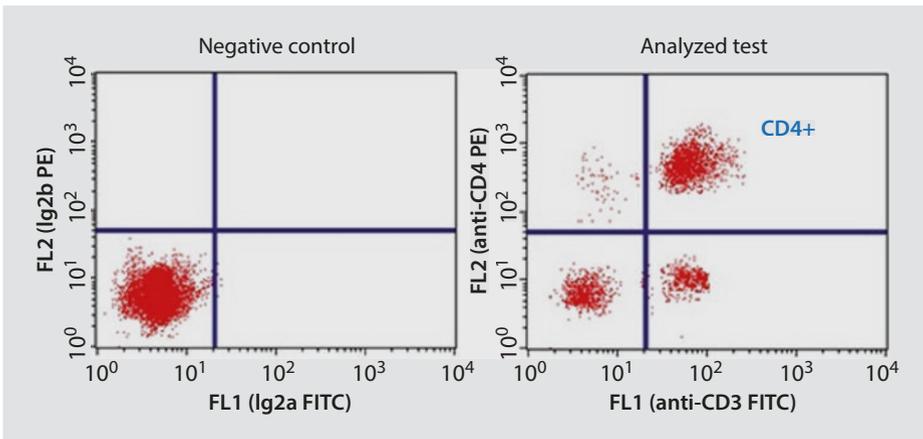
■ Fig. 5.1 Flow cytofluorometry

samples. The fluorescence light is often employed to report the densities of specific surface receptors on cells, distinguish subpopulations of differentiated cell types, and assess the intracellular components of cells including total DNA, specific nucleotide sequences in DNA or mRNA, newly synthesized cytokines, and the quantities of other specific components of the cells. The light signals are collected by lenses, focused onto a forward scatter detector, side scatter detector, and fluorescence photomultiplier detectors, amplified, and measured. Previously labeled by fluorescent dyes, or fluorochromes (fluorophores), monoclonal antibodies to different cell markers enable the revealing of these markers. The fluorochrome-labeled molecules are distinguished from each other by different colors, which appear under fluorescence photomultiplier detectors depending on the chosen fluorochrome and certain technical conditions including detection wavelength, etc.

There are samples of FCFM histograms, or plots, as follows (see ■ Figs. 5.2 and 5.3).



■ Fig. 5.2 FCFM plots



■ Fig. 5.3 FCFM plots

Modern flow cytometers are capable of analyzing several thousand particles every second, in real time, and can simultaneously sort them and isolate those with specified markers.

### 5.3 Enzyme-Linked Immunosorbent Assay (ELISA)

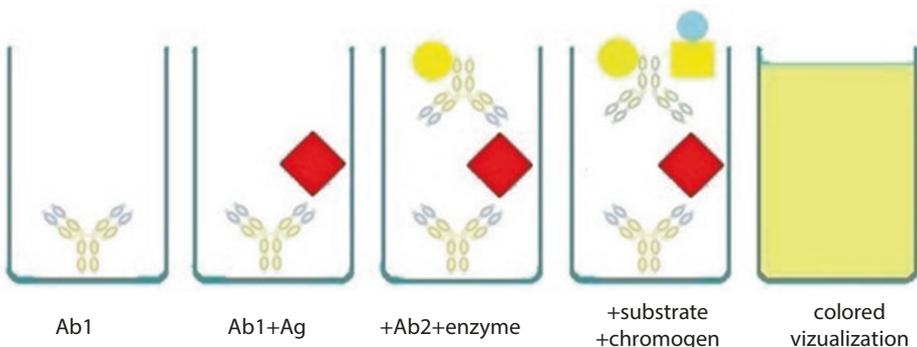
#### Definitions

*Enzyme-linked immunosorbent assay (ELISA)* is the technique based on the visualization of binding antigen or antibody by a linked enzyme that converts a colorless substrate into a colored reaction.

This method uses for the sensitive and specific measurement of any proteins. A monoclonal antibody (Ab1) specific for the particular antigen (Ag) is previously absorbed onto microwells (see ■ Fig. 5.4). If this antigen is present in a sample, it will be bound and immobilized. In contrast, other antigens will be removed upon a wash step of the assay.

A second monoclonal antibody (Ab2 + enzyme) directed against the antigen and conjugated to an enzyme (e.g., horseradish peroxidase) is then added. As a consequence, Ab1/Ag/Ab2 + enzyme sandwich-like complex is formed and immobilized on the microwell wall. At this moment, the substrate plus chromogen may be placed into the microwell to result in a colored visualization, which can be quantified photometrically and is proportional to the amount of Ab2 and hence to the concentration of the antigen.

Currently, this method has many modifications (direct ELISA, sandwich ELISA, ELISPOT, NU-ELISA, etc.) and may also be used as the visualization system for other techniques.



■ Fig. 5.4 Enzyme-linked immunosorbent assay (ELISA)

## 5.4 Immunoblot (Western Blot)

### Definitions

*Immunoblot (Western blot)* is the technique based on the separation of a mixture of various proteins by gel electrophoresis, transfer of them by blotting to a nitrocellulose membrane and detection of a specific protein by labeled antibodies. *Eastern* and *Northern blots* operate with DNA and RNA and refer to the molecular biological methods.

5

The method is employed to detect specific proteins in a mix of different proteins in a sample of tissue homogenate (e.g., for precise diagnostics of *HIV* infection). Proteins are first separated in a sodium dodecyl sulfate (SDS)-polyacrylamide gel. Then, in a blotting step, the proteins are transferred to a sheet of nitrocellulose membrane. The gel containing the separated proteins is placed on the membrane.

Electrophoresis accomplishes protein transfer. In the electric field, the proteins coated with negatively charged SDS migrate toward the positive electrode. As the proteins migrate out of the gel, they are captured on the membrane because nitrocellulose binds any protein that contacts it.

The proteins stick to the membrane in the same positions they had in the gel. Thus, the membrane is a “blot” of the gel. A primary antibody, chosen to specifically bind only the protein of interest, is then added. After the washing step, the primary antibody that is bound to the protein of interest remains only.

Several methods of visualization (e.g., ELISA) are used to detect the antibody-labeled protein. They all use any secondary antibody, which binds specifically to the Fc fragment of the primary antibody.

## 5.5 Radioimmunoassay (RIA)

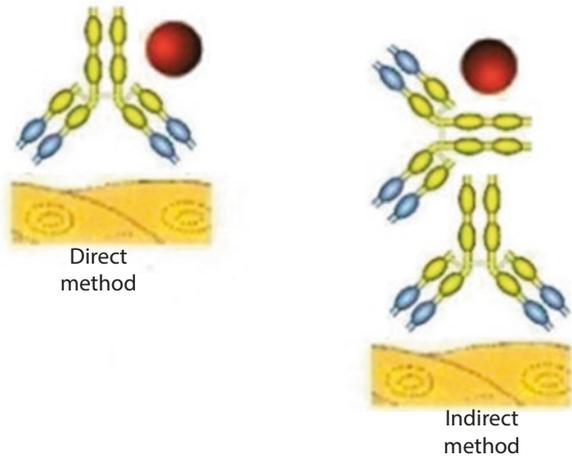
The method developed by 1977 Nobel Laureate R.S. Yalow for the extremely sensitive and specific measurement of any proteins by the competition of an analyte (unlabeled antigen) in a serum sample, with the same antigen, labeled radioactively ( $^{125}\text{I}$ ), for a fixed quantity of antibody *in vitro*. If a serum level of the analyte is low, the precipitate will indicate a high radioactive count and vice versa. RIA has many uses, including blood bank screening for viruses, measurement of concentrations of hormones, cytokines, chemokines, neurotransmitters, drug detection, etc. The radioactivity measurement is made using a gamma scintillation counter.

## 5.6 Immunohistochemistry Staining

### Definitions

*Confocal laser scanning microscopy* is the fluorescence microscopy, which allows the generation of multilayer high-resolution images of analyte's components when the analyte may remain alive.

■ Fig. 5.5 Immunohistochemistry staining



Immunohistochemistry staining makes antigen-antibody complexes visible under a fluorescence microscope or in laser-light flow; hence it is mainly used for immunohistochemistry staining and flow cytometry.

Using the *direct method*, a less sensitive, the fluorescent dye (e.g., fluorescein isothiocyanate = FITC)-labeled primary monoclonal antibody reacts with the antigen in the tissue (see ■ Fig. 5.5). Using the *two-step indirect method*, a higher sensitive, labeled secondary antibody reacts with an unlabeled primary antibody bound to the antigen in the tissue. There are many multistep method variants, including the ELISA technique, e.g., with the use of biotin/avidin/enzyme complex. Avidin, a bridge for fluorochrome and enzyme, has a high affinity for biotin, which, in turn, can biotinylate antibodies.

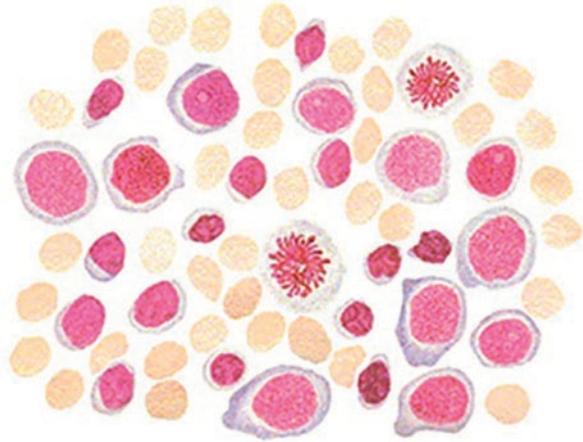
*Confocal (widefield) fluorescence microscopy*, or *confocal laser scanning microscopy*, an up-to-date form of the fluorescence microscopy, allows the generation of multilayer high-resolution images of tissues, cells, cellular organelles, and even molecules stained with multiple fluorescent probes. The technique enables to evaluate any biological analyte in progress, under both physiological and pathological conditions as this analyte may remain alive. For example, quantification of NETosis and other immunological processes can be achieved by means of confocal laser scanning microscopy. Nowadays, there are many applications of the technique in clinical medicine. The cornea as a transparent tissue became one of the first model organs for the examination by confocal laser scanning microscopy. This technique also uses for the evaluation of stem cell-based cardiac regenerative therapy, for the diagnosis of various skin diseases including dermatitis and cancers, GI tract diseases, etc.

## 5.7 Cell Proliferation Assays

### Definitions

*Mitogens* are very potent stimulators of lymphocyte proliferation independent of their antigenic specificity.

■ **Fig. 5.6** Lymphoblasts in the lymphoblast transformation test (LTT)



A classical cell proliferation assay, lymphocyte transformation test (LTT), measures the proliferation of lymphocytes in response to any antigen *in vitro*. This concept of LTT has been confirmed by the generation of antigen-specific T-cell clones. The method is based on the ability of sensitized lymphocytes to turn into lymphoblasts under the influence of the antigen or nonspecific mitogens *in vitro* (see ■ Fig. 5.6). In fact, the method measures the clonal expansion of lymphocytes.

The mitogens are compounds capable of inducing mitosis. There are many mitogens for lymphocytes, for example, Gram-negative bacterium *lipopolysaccharide* (LPS) for B cells, *pokeweed mitogen* (PWM) for both T cells and B cells, and *phytohemagglutinin* (PHA) and *concanavalin A* (ConA) for T cells.

Diluted heparinized blood is placed in a special medium for blood cell separation (e.g., Ficoll-Hypaque) at a density gradient of 1077 and centrifuged. As a consequence, different fractions are formed: diluent, lymphocytes + monocytes mix, medium, and erythrocytes + granulocytes mix. The lymphocytes + monocytes mix are drawn up; from this mix monocytes are removed by means of their adhesion to dishes, and the pure lymphocytes are next added into a special cultivation culture with a stimulus, which must be assessed and left for 4–5 days at 37 °C. The lymphoblasts may be counted by radioactive nucleoside  $^3\text{H}$ -thymidine's label in a scintillation counter as  $^3\text{H}$ -thymidine is incorporating into the new strands of DNA (but not RNA) during the cell division.

However, this *radioactive technique* does not allow to assess the response of separate subsets and activation-induced cell death in cell cultures. To solve the difficulty, *colorimetric proliferation assays* with the use of dyes MTT and MTS (MTT and MTS assays) and *cytofluorometric proliferation methods* in which lymphocyte division markers such as carboxyfluorescein succinimidyl ester (CFSE) may be used.

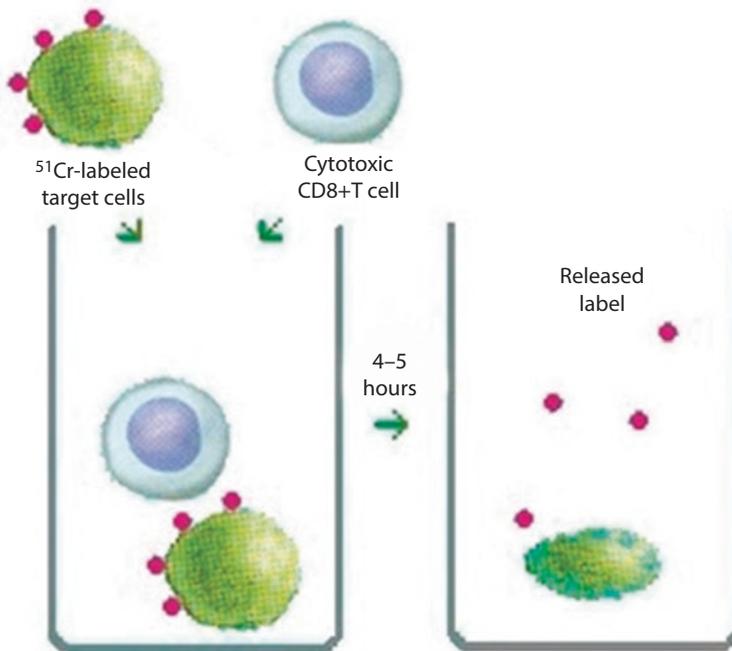
Tests, which assess the cell proliferation (e.g., assessment of Ki-67 nuclear protein), are widely employed in cancer research. Commonly, received data are often evaluated by the different visualization methods and may exploit reverse transcription polymerase chain reaction (RT-PCR).

## 5.8 Cytotoxicity Assays

### Definitions

*Cytotoxicity* is a term, which may mean (1) a kind of the effector activity of cells and molecules and (2) a method for assessing killing effects of the cells, molecules, and tested compounds.

This method is based on the incubation of effector cells such as cytotoxic CD8 + T cells and/or NK cells and  $^{51}\text{Cr}$ -labeled target cells together and evaluation of damage to the targets by measurement of radioactivity in the culture fluid (see ■ Fig. 5.7). The appropriate HLA-matched cells from the same patient (e.g., virus-infected cells) are used as target cells for cytotoxic CD8 + T cell assessment, whereas a cell line highly susceptible to NK-cell action (e.g., erythroleukemia cell line K562) is used for the evaluation of NK-cell-mediated cytotoxicity.



■ Fig. 5.7 Cytotoxicity assay

## 5.9 Tests on Phagocytosis

5

*Chemotaxis* of neutrophils and monocytes may be assessed *in vitro* using a multiwell Boyden-like chamber and 0.75–5  $\mu\text{m}$  micropore-sized filter. The cells migrate through the filter with a certain speed under the influence of chemoattractants, which are present in the low department. The data may be read as a cell speed using a “leading front,” the distance between the start line and the forward cell, divided into a time unit. Another method, employed *in vivo*, involves the use of a so-called skin window, in which a skin area is scarified and covered by a slide or skin chamber (see ■ Fig. 5.8). In the first case, neutrophils adhere to the slide within 6 h, whereas monocytes attach to it within 24 h. In the second case, only neutrophils migrate into the chamber; specifically, a skin cell pool moves on within 6 h, and a circulating cell pool enters within 24 h. The skin chamber technology also enables the detection of various molecules in skin exudate.

The *cell adhesion molecules* on the neutrophil surface such as CD18 (a part of LFA-1) and sialylated Lewis<sup>x</sup> (CD15), essential for diagnosing leukocyte adhesion defects, may be assessed by flow cytometry.

In order to assess the *opsonization* and *endocytosis*, test microbes or latex particles are used. Test microbes are labeled by fluorochrome and opsonized with immunoglobulin and complement of pooled sera. By utilizing both opsonized and nonopsonized microbes, which are also available, both opsonization capacity and endocytosis can be measured at the same time.

The nitroblue tetrazolium test (NBT test) is a simple screening method to assess the oxygen-dependent *cytotoxicity* of neutrophils. As a consequence of “the respiratory burst,” yellow dye nitroblue tetrazolium is converted to a blue precipitate, formazan, which is deposited within the cell cytoplasm (see ■ Fig. 5.9). The higher the blue score, the better the cell is at producing reactive oxygen species (ROS). The normal value does not exceed 10%. The NBT test may be carried out with stimulation of *C. albicans* or other test microbe/test particles.

A *respiratory burst* of phagocytes may also be evaluated by lucigenin-dependent chemiluminescence, for superoxide anion, and by luminol-dependent chemiluminescence, for singlet oxygen, using a luminometer. The NBT test and chemiluminescence employ for diagnosing chronic granulomatous disease, neutrophil G6PD deficiency, and similar syndromes of primary immunodeficiencies.

■ Fig. 5.8 Skin chamber



■ Fig. 5.9 Nitroblue tetrazolium test (NBT Test)



## 5.10 Molecular Biological Methods

### Definitions

*Deoxyribonucleic acid (DNA)* is commonly a double-stranded nucleic acid (dsDNA), which consists of two strands, a sense strand and antisense strand, complementary to each other to constitute the double helix. The sense strand is encoding that runs from 5' to 3' end. Conversely, the antisense strand runs from 3' to 5' end. DNA contains two types of nucleotide base pairs (bps) in which adenine (A) is complementary to thymine (T) and cytosine (C) is complementary to guanine (G). Each base pair is stabilized by the hydrogen bonds.

*Genes* are DNA segments encoding the synthesis of whole proteins or single polypeptide chains.

*Ribonucleic acid (RNA)* is another nucleic acid that is commonly single-stranded (ssRNA) and contains uracil (U) instead of thymine (T). Messenger RNA (mRNA) plays a role in the protein synthesis and occurs during the *gene expression*. The mRNA takes the DNA antisense strand as its template and is correspondingly the copy of the DNA sense strand. Initially, immature pre-mRNA includes introns, and then it turns through processing into mature RNA, which is ready for translation. *Splicing* is the exclusion of introns from pre-mRNA.

*Molecular biological methods* include *Southern* and *Northern blots*, *restriction fragment length polymorphism (RFLP)*, *polymerase chain reaction (PCR)*, *reverse transcription PCR (RT-PCR)* and their modifications, and *gene DNA chip technology*.

Nowadays, there is a variety of protocols of the molecular biological techniques, which is used in both immunogenetics and sister fields. Analogous to antigen-antibody reactions, the molecular biological methods are based on the interactions of complementary nucleic acids (cDNA and cRNA). Target DNA (analyte) is commonly dsDNA, which upon the denaturation becomes ssDNA. Two formed ssDNA are complementary to each other, but they both contain unknown sequences of nucleotides.

Hybridization, a method of the detection, represents the precise ligation of nucleotides of the target ssDNA to cDNA or cRNA with known nucleotide sequences labeled by a radioactive element, fluorochrome, or enzyme for visualization. This label is usually termed as a probe. The target nucleotide sequence may also be read using gel electrophoresis and directly by sequencing.

The *blotting*, Southern and Northern blotting, is an ancillary technique by which target nucleic acid, analogous to proteins during the Western blotting, may be electrophoretically transferred from the liquid phase to the solid nitrocellulose membrane.

Analyte molecules first are separated by size using polyacrylamide gel electrophoresis and then also transferred electrophoretically directly onto solid nitrocellulose membrane for hybridization to cDNA. After that, the film is washed away, to remove any unbound cDNA.

Since nucleotide sequences in the cDNA are common, the blots may be then detected by a certain method of visualization (see ■ Table 5.1).

*Restriction fragment length polymorphism (RFLP)* is used for detection of molecular anomalies (mutations) in genes, mapping the human chromosomes, and identification of persons including forensic use. The technique exploits cleavage of the DNA strand with restriction enzymes, which target a specific DNA recognition sequence.

The classical principle of *polymerase chain reaction (PCR)* according to 1993 Nobel Laureate K. Mullis is composed in the multifold copying of target DNA with the use of *primers* through their polymerization by DNA polymerase to amplify billions of copies (*amplicons*) required for the detection of the target gene. Analyte must contain the well-characterized target gene with the target sequence of nucleotides (on dsDNA). Primer is

■ Table 5.1 Blotting techniques

Method	Analyte	Detection	Possibilities
Southern blot (developed by E.M. Southern)	DNA	DNA hybridization to a radioactive (or other) cDNA probe	Detection of a particular gene of interest in genomic DNA
Northern blot	RNA	RNA hybridization to a radioactive (or other) cDNA probe	RNA isolation from a different tissue to see which tissue expresses a certain gene (e.g., in primary immunodeficiencies)
Western blot	Proteins (antigens)	Binding to antibodies conjugated to any label	Detection of a particular protein

a short piece of DNA (20–30 bps) synthesized *in vitro*, which is complementary to this target sequence. The pair of the primers includes a forward primer and different reverse primer, but each primer is complementary to the 3' ends of each of the target sequence on both sense and antisense strand of the DNA. Primers will be annealed and extended to form then dsDNA under the influence of thermostable *Thermus aquaticus* (*Taq*) or *Pyrococcus furiosus* (*Pfu*) DNA polymerase in an amplifier (or thermocycler). The amplifier is a thermostat in which heat regime can be changed automatically and copying cycles can be repeated. Polymerase starts synthesizing new DNA strand complementary to the target sequence from the end of each primer.

Any PCR cycle includes:

1. DNA denaturation, or separation of strands (at 94 °C) to break all H bonds and get ssDNA
2. Annealing of the pair primers (at 40–60 °C) to find and bind complementary sequence on each ssDNA
3. Elongation of the pair primers (at 70–74 °C) by DNA polymerase extension

Such cycles repeat 20–30 times. As a result of amplification, after the first few cycles, there are formed excessively long sequences. After that, since the primers bind to their targets on these long sequences, they begin to shorten until most of the amplicons in the mixture reaches the expected length of the target gene. The nucleotide sequence in these copies (amplicons) may be identified by sequencing, gel electrophoresis, hybridization, or other methods. Hybridization allows performing the detection by a radioactive element, fluorochrome, or enzyme (ELISA). For the intermediate bridge, biotin and avidin (or streptavidin) may be used as they have the strong ability to bind up to each other. Also, internal control (or internal standard) is required in any cases.

As contrasted to conventional PCR, *real-time PCR* operates the amplification of a targeted DNA molecule during the PCR, not at its end, that allows this modification to use as a quantitative or semiquantitative approach. In addition, there is *quantitative PCR* (*qPCR*).

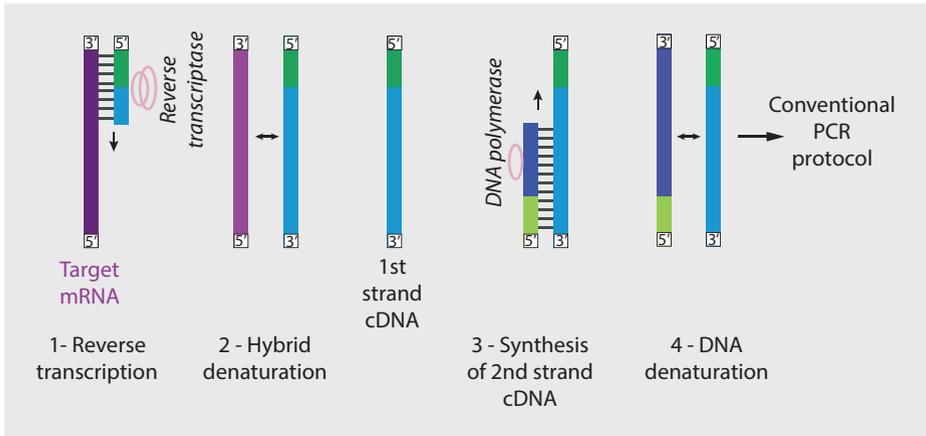
PCR is used for (1) selective DNA isolation and cloning genes; (2) detection of mutations; (3) HLA typing for transplantation; (4) disputed paternity and person's identification (including forensic use); (5) infection diagnostics except for RNA viruses; (6) scientific research of ancient sources, etc.

PCR modification, *reverse transcription PCR* (*RT-PCR*), implies extraction of mRNA from cellular suspension, synthesis of cDNA by *reverse transcriptase* (*RT*), and further common protocol (see  Fig. 5.10).

This quantitative and in fact functional technique allows the diagnosis of RNA viral infections such as *HIV* and *hepatitis C virus* and analysis of mRNA transcripts such as those produced by the synthesis of molecules of the immune system. A well-known limitation to RT-PCR is the RNAase activity. Assessment of the gene expression and correspondingly mRNA by Northern blot and RT-PCR differs by details of received information. However, RT-PCR is more sensitive.

Combination of PCR and RFLP (*PCR-RFLP analysis*) is used for the detection of intraspecies and interspecies variations, e.g., when there is a single nucleotide polymorphism.

*Gene DNA chip* (or *DNA microarray*) technology based on a collection of small DNA spots attached to a solid surface is a tool for effective simultaneous analysis of



■ Fig. 5.10 Reverse transcription PCR (RT-PCR)

the enormous number of human genes including those encoded immune system's molecules. Analogous to computer microchips (photolithography), the technique enables to synthesize in vitro lots of different small pieces (30 bps) of DNA (or oligonucleotides) on a single chip. The oligonucleotides are designed to detect mRNA from a significant amount of genes using their hybridization with known sequences, biotin and streptavidin-fluorochrome labeling, and laser scanner reading the probes.

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

Molecular biological methods are based on complementary interactions of:

1. An antigen and antibody.
2. Amino acid residues.
3. Nucleic acids.
4. Polysaccharides.

#### ? Question 2

Target dsDNA if denaturalized becomes:

1. ssDNA.
2. ssRNA.
3. dsRNA.
4. Amino acid.

#### ? Question 3

Hybridization is precise ligation of target ssDNA to:

1. Denaturalized dsDNA.
2. dsRNA.
3. Amino acid.
4. cDNA or cRNA with labeled known sequences.

**? Question 4**

The probe is:

1. A labeled sequence for visualization.
2. A primer.
3. An amplicon.
4. Any analyte.

**? Question 5**

A primer is:

1. Any nucleotide sequence.
2. The known nucleotide sequence complementary to the target gene.
3. The target gene.
4. The unknown nucleotide sequence complementary to the target gene.

**? Question 6**

An amplicon is:

1. DNA fragment formed due to restrictase activity in RFLP.
2. A product of Southern blot.
3. Formed copy of a sequence in the course of PCR.
4. A product of Northern blot.

**? Question 7**

This method is not related to molecular biological:

1. Restriction fragment length polymorphism (RFLP).
2. Gene DNA chip technology.
3. Flow cytometry.
4. Polymerase chain reaction (PCR).

**? Question 8**

This method is related to molecular biological:

1. Northern blot.
2. Radioimmunoassay (RIA).
3. Lymphoblast transformation test (LTT).
4. Flow cytometry.

**? Question 9**

The enzyme important for amplification cycles in PCR is:

1. Restrictase.
2. Taq DNA polymerase.
3. Reverse transcriptase.
4. Phospholipase C.

**? Question 10**

Southern blot allows:

1. Isolation of a particular protein.
2. Detection of a certain gene.
3. Evaluation of certain gene expression.
4. Measurement of phagocytosis.

**? Question 11**

Northern blot enables:

1. Detection of a certain protein.
2. Detection of a particular gene.
3. Measurement of chemotaxis.
4. Assessment of certain gene expression.

**? Question 12**

PCR is widely spread in immunogenetics and sister fields:

1. Unknown.
2. Doubtfully.
3. Yes.
4. No.

**? Question 13**

PCR-RFLP is commonly used for:

1. Visualization in Southern blot.
2. Detection of a single nucleotide polymorphism.
3. Simultaneous analysis of enormous number of human genes.
4. Detection of a particular protein.

**? Question 14**

Reverse transcription polymerase chain reaction (RT-PCR) allows:

1. Measurement of chemotaxis.
2. Evaluation of gene expression.
3. Measurement of chemiluminescence.
4. Detection of a certain gene.

**? Question 15**

Visualization is important for any molecular biological technique too:

1. Certainly.
2. Unknown.
3. No.
4. Does not matter.

**? Question 16**

DNA microarray technology enables:

1. Cleavage of DNA strands with restrictase.
2. Evaluation of antigen-antibody reactions.
3. Measurement of phagocytosis.
4. Simultaneous analysis of a large amount of genes.

## 5.11 Clinical Assessment of Immunoassays

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You can see a complex of the immunoassays in ■ Table 5.2.

**Table 5.2** Parameters of immunoassays

Parameter (the blood)	Normal value
WBC ( $10^9/L$ )	4000–9000
Lymphocytes (%)	19–37
Lymphocytes ( $10^9/L$ )	1200–3000
CD3+ T cells (% of lymphocytes)	55–80
CD3+ T cells ( $10^9/L$ )	800–2200
Helper CD4+ T cells (% of lymphocytes)	31–51
Helper CD4+ T cells ( $10^9/L$ )	600–1600
Cytotoxic CD8+ T cells (% of lymphocytes)	19–40
Cytotoxic CD8+ T cells ( $10^9/L$ )	300–800
Index CD4+ / CD8+	1.0–2.5
HLA-DR+ (% of lymphocytes)	5–20
CD19+ B cells (% of lymphocytes)	5–19
CD19+ B cells ( $10^9/L$ )	100–500
Spontaneous lymphoblast transformation test (LTT)	500–1500
Response to phytohemagglutinin (PHA)	20,000–80,000
Index of stimulation	20–75
Response to pokeweed mitogen (PWM)	5000–15,000
Index of stimulation	5–25
NK-cell activity (%)	>25
CD3 + CD4 + CD8+ (% of lymphocytes)	<2
CD3-CD16 + CD 56+ NK cells (% of lymphocytes)	6–20
CD3-CD16 + CD 56+ NK cells ( $10^9/L$ )	0–300
CD3 + CD16 + CD 56+ NK cells (% of lymphocytes)	<10
Parameter (the blood)	Normal value
CD3 + CD16 + CD 56+ NK cells ( $10^9/L$ )	150–600
Naive IgD + CD27- B cells (% of B cells)	43–82
Naive IgM + CD27- B cells (% of B cells)	43–82
IgD + CD1cCD27+ marginal zone B cells (% of B cells)	7.5–32.5
IgM + CD1cCD27+ marginal zone B cells (% of B cells)	7.5–32.5

(continued)

**Table 5.2** (continued)

Parameter (the blood)	Normal value
IgD-IgM + IgG + IgA + CD27+ memory B cells (% of B cells)	6.5–29
IgD + CD38++ transitional B cells (% of B cells)	0.6–3.4
IgM + CD38++ transitional B cells (% of B cells)	0.6–3.4
IgM-CD38+++ plasmablasts (% of B cells)	0.4–3.6
CD21 <sup>lo</sup> CD38- activated B cells (% of B cells)	0.9–7.6
BAFF-R B cells (% of B cells)	>95
Phagocytic index for neutrophils, <i>S. aureus</i>	95–99
Phagocytic index for monocytes, <i>S. aureus</i>	85–95
Nitroblue tetrazolium test (NBT test) (% of neutrophils)	5–12
IgM (g/L)	0.8–2.5
IgG (g/L)	9.0–18.0
IgA (g/L)	1.0–3.5
IgE (IU/mL)	<130

5

**From a clinical point of view,** a wide range of medical tests, immunoassays, requires proper clinical evaluation depending on each case in the individual context.

1. The evaluation must be of all parameters, taking into account the relative and absolute quantities of molecules and cells of interest in the blood.
2. The assessment must be based on a clinical case, on an individual patient at a certain age, on a disease characterized by such qualities as form, period, and severity of the pathologic process.
3. Preferences must be given to extended variables over small variables.
4. Evaluation of the patient must be repeated during the course of the disease.
5. Priority must be given to the clinical approach over the laboratory approach.

### Key Points

1. There are lots of various techniques exploited in fundamental and clinical immunology and allergy. If there are any clinical or scientific studies carried out in patients, the results should be evaluated from a clinical point of view, i.e., in the reference to each patient.
2. To avoid the diagnosis mistakes, any test used in clinical immunology and allergy should have very high sensitivity and specificity.

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# Immunopathology

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**Electronic supplementary material** The online version of this chapter ([https://doi.org/10.1007/978-3-030-03323-1\\_6](https://doi.org/10.1007/978-3-030-03323-1_6)) contains supplementary material, which is available to authorized users.

## Learning Objectives

*Knowledge.* Upon successful completion of the chapter, students should be able to:

1. List the groups of immunopathology.
2. Be familiar with the interpretation of clinical and laboratory data and interventional strategies as it relates to primary immunodeficiencies.
3. Draw the secondary immunocompromised conditions including *HIV/AIDS*.
4. Describe type I–IV hypersensitivity and the clinical manifestations of common atopic and allergic disorders.
5. Define the breakdown of autotolerance, and clinical and laboratory data related to the autoimmune conditions. Distinguish between the autoimmune and autoinflammatory disorders.
6. Recognize the fundamental mechanisms of cancer immunopathogenesis.
7. Identify the basic principles of graft rejection and graft-versus-host disease (GVHD).

*Acquired Skills.* Upon successful completion of the chapter, students should demonstrate following skills, including:

1. Interpret the knowledge related to immunopathology.
2. Critically evaluate the clinical literature about immune-mediated diseases.
3. Discuss the scientific articles from the current research literature to criticize experimental and clinical data and formulation of new hypotheses in clinical immunology/allergy.
4. Obtain a patient's history including history of present illness; past medical history; social, family, and occupational history; and review of systems.
5. Perform a patient's physical examination in a thorough manner.
6. Explain the rationale for choice of interventional immunology in a patient.
7. Attain a clear perception of the presented immunology definitions expressed orally and in written form.
8. Formulate the presented immunology terms.
9. Correctly answer quiz questions.

*Attitude and Professional Behaviors.* Students should be able to:

1. Have the readiness to be hardworking.
2. Behave professionally at all times.
3. Recognize the importance of studying and demonstrate a commitment.
4. Demonstrate the consideration of the patient's feelings, ethnic, religious, cultural, and social background and be able to display empathy.

## 6.1 Introduction

---

The immune system can suffer from some diseases such as primary immunodeficiencies, secondary immunocompromised conditions including *HIV* infection, allergic diseases, autoimmune/autoinflammatory disorders, and cancer. The immune system is responsible for graft rejection and/or survival. The reader can find the information on the development of diagnostic, therapeutic, and preventative strategies to combat this threat and an odd concept concerning atopic allergic conditions.

Diseases of the immune system are based on the reactivation of opportunistic microbes, a breakdown in the natural tolerance to self-antigens and allergens, and/or tumor transformation of the immune and other cells.

There are four groups of immunopathology:

1. *Immunodeficiencies* – the low functional activity of the immune system:
  - Primary immunodeficiencies (hereditary and innate)
  - Secondary or acquired immunodeficiencies (immunocompromised conditions), including *HIV/AIDS*
2. *Allergic diseases* – when the immune mechanisms are involved. They may be divided into four types of reactions (according to Gell and Coombs):
  - Atopic IgE-dependent diseases (type I)
  - Cytotoxic disorders (type II)
  - Immune complex-mediated disorders (type III)
  - Delayed T-cell-mediated hypersensitivity disorders (type IV)
3. *Autoimmune diseases* are caused by a breakdown in the natural tolerance to autoantigens, and *autoinflammatory disorders* originate from innate immunity imbalances.
4. *Immunoproliferative neoplasms* – leukemias, lymphomas, lymphosarcoma, etc. Any cancer may be in part related to immunopathology.

## 6.2 Immunodeficiencies

---

### Definitions

*Immunodeficiency* is a state in which the ability of the immune system to protect the body against pathogens and cancer is decreased or completely absent. The *primary (hereditary/innate) immunodeficiencies* are caused by gene mutations, whereas *secondary immunocompromised conditions* may occur due to the influence of harmful environmental and endogenous factors.

Molecular anomalies in the genes of some receptors, cytokines, signaling molecules, and enzymes of lymphocytes and other cells transmitted by heredity lead to extremely decreased functions of innate and adaptive immunity, *primary immunodeficiencies*, and *major primary immunodeficiencies*. However, some gene's anomalies do not result in fatal consequences and may be termed as “minor” primary immunodeficiencies. Primary immunodeficiency diseases number at least 176 hereditary disorders that are thought to be individually rare. The frequency of occurrence of primary immunodeficiencies is estimated to be  $10^4$ , but the actual prevalence and incidence of these diseases and syndromes remain unclear. For example, for Europe, only about 15,000 cases were registered (2.27%) to 2013, whereas the upper estimate was 638,000 cases. Therefore, proper epidemiologic studies are required. For the precise diagnosis of any primary

immunodeficiency, the correct molecular biological and genetic tests might need to be ordered because certain gene anomalies would have to be revealed. These techniques are the Northern blot, restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR), etc.

Some mutations have already been mentioned in chapters devoted to innate mechanisms and adaptive immune responses. You can see samples of primary immunodeficiencies in ■ Table 6.1 as well as any information about gene mutations, which are linked to the primary immunodeficiencies on Online Mendelian Inheritance in Man® (OMIM) at ► [www.omim.org](http://www.omim.org).

■ Table 6.1 Samples of “major” primary immunodeficiencies

Affected cells	Name	Gene's anomaly	Clinical symptoms
T cells and B cells	X-linked <i>severe combined immunodeficiency (SCID)</i>	IL2RG on Xq13.1, which encodes $\gamma$ chain (CD132) receptor for IL2 as well as IL4, IL7, IL9, IL12, IL13, and IL15	Very severe infections from the birth (“bubble boy disease”)
T cells and B cells	<i>SCID</i> , reticular dysgenesis	AK2 (mitochondrial adenylate kinase 2) on 1p35.1 that leads to disability to hemopoiesis	Very severe infections from the birth, cytopenias
T cells and B cells	<i>SCID</i> , adenosine deaminase deficiency	ADA (adenosine deaminase) on 20q13.12 that leads to disorder of lymphopoiesis	Very severe infections from the birth or later
T cells and B cells	<i>SCID</i> , type I bare lymphocyte syndrome	TAP1/TAP2 on 6p21.32	Severe infections of respiratory tract in babies, toddlers, and older children
T cells and B cells	<i>SCID</i> , type II bare lymphocyte syndrome	Different regulatory genes on 1q21.3, 13q13.3, 16p13.13, and 19p13.11, which are required for transcription of HLA II genes	Very severe infections from the birth or later
T cells	<i>SCID</i> , T-cell-negative, B-cell-positive, NK-cell-positive	IL7RA on 5p13.2 that results in disorder of signaling through IL7R $\alpha$ (CD127) and blockade in T lymphopoieses	Very severe infections from the birth
T cells	<i>SCID</i> , T-cell-negative, B-cell-positive, NK-cell-negative	JAK3 on 19p13.11 that leads to blockade in T-cell and NK-cell development	Very severe infections from the birth

■ **Table 6.1** (continued)

Affected cells	Name	Gene's anomaly	Clinical symptoms
T cells	Immunodeficiency 17/19	CD3G/ CD3D on 11q23.3 that leads to disorder of T lymphopoiesis and decrease in CD3 $\gamma\delta$	Recurrent infections in babies, toddlers, and later
T cells	Immunodeficiency 48	ZAP70 on 2q11.2 that leads to selective disorder of CD8+ T-cell lymphopoiesis	Recurrent infections in babies, toddlers, and later
B cells	<i>SCID</i> , Omenn syndrome	RAG1/RAG2 on 11p12 that leads to disorder of B lymphopoiesis and partially T lymphopoiesis	Early infections, dwarfism, reticuloendotheliosis with eosinophilia, predisposition to tumors
B cells	X-linked <i>agammaglobulinemia</i> ( <i>Bruton's syndrome</i> )	BTK (Bruton's tyrosine kinase) on Xq21.3-q22	Recurrent pyogenic infections in male babies, underdeveloped secondary lymphoid organs
B cells	<i>Common variable immunodeficiency (CVID)</i> (heterogeneous group)	ICOS on 2q33.2 (CVID1), TNFRSF13B on 17p11.2 (CVID2), CD19 on 16p11.2 (CVID3), TNFRSF13C on 22q13.2 (CVID4), CD20 (MS4A1) on 11q12.2 (CVID5), CD81 on 11p15.5 (CVID6), CD21 (CR2) on 1q32.2 (CVID7), etc.	Recurrent sinopulmonary infections at any age, increased incidence of autoimmune disorders and tumors
B cells	<i>Hyper-IgM syndrome</i> , types 1–5	Different mutations: TNFSF5 on Xq26.3 (type 1), AICDA on 12p13.31 (type 2), CD40 on 20q13.12 (type 3), unknown (type 4), and UNG on 12q24.11 (type 5), which lead to inability of B cells to undergo antibody isotype switching	Recurrent pyogenic infections in babies, underdeveloped secondary lymphoid organs, autoimmune cytopenias
<b>Combined</b>	Wiskott-Aldrich syndrome	WASP (Wiskott-Aldrich syndrome protein) on Xp11.22-p11.23 that leads to cytoskeleton defect	Clinical triad: (1) Increased susceptibility to infections in babies and toddlers (2) Thrombocytopenic purpura (3) Mild eczema as well as increased incidence of tumors

(continued)

**Table 6.1** (continued)

Affected cells	Name	Gene's anomaly	Clinical symptoms
<i>DNA defects</i>	DiGeorge syndrome	1.5–3.0 Mb deletion of chromosome 22q11.2 including genes responsible for physical malformations	Seizures at the birth because of hypocalcemia (parathyroid hypoplasia), thymic hypoplasia, derivatives of the pharyngeal arches, congenital heart defects, etc.
<i>DNA repair defect</i>	Ataxia telangiectasia (Louis-Bar syndrome)	ATM (ataxia telangiectasia mutated gene) on 11q22.3 that is responsible for cell cycle disorder	Clinical triad: (1) Progressive cerebellar ataxia (2) Oculocutaneous telangiectases, (3) Chronic sinopulmonary infections as well as increased incidence of tumors
<i>DNA repair defect</i>	Nijmegen breakage syndrome	NBN (nibrin gene) on 8q21.3 responsible for cell cycle disorder	Microcephaly, growth retardation, early infections, and predisposition to cancer
Many cells	Immunodeficiencies 27A/27B	IFNGR1 (CD119) on 6q23.3 that leads to defects of phagocytosis and development of inflammation in response to <i>Mycobacteria</i> , and different pathogens	Disseminated BCG infection, SCID-like and CGD-like symptoms
NK cells and other cells	Immunodeficiency 20	CD16 (FCGR3A) gene on 1q23.3 that leads to decreased NK-cell-mediated cytotoxicity	Recurrent viral infections <i>Epstein-Barr virus (EBV)</i> , <i>herpes zoster virus (HZV)</i> and other herpesviruses, and <i>human papilloma viruses (HPV)</i>
Neutrophils	X-linked <i>chronic granulomatous disease (CGD)</i>	CYBB on Xp21.1-p11.4, which encodes p22phox for phagocyte oxidase that leads to inability to generate ROS	Abscesses of the skin, lymph nodes, and liver in male babies
Leukocytes	Type 1 <i>leukocyte adhesion deficiency (LAD1)</i>	ITGB2 (CD18) on 21q22.3 that leads to inability to synthesize CD18 (a $\beta$ unit of integrin)	Repeated bacterial and fungal infections, intestinal or perianal fistulae, delayed wound healing, and high leukocytosis

■ **Table 6.1** (continued)

Affected cells	Name	Gene's anomaly	Clinical symptoms
Leukocytes	Type 2 <i>leukocyte adhesion deficiency</i> (LAD2)	SLC35C1 on 11p11.2 that results in the inability to synthesize sialylated Lewis <sup>x</sup>	Repeated bacterial and fungal infections, intestinal or perianal fistulae, delayed wound healing, and high leukocytosis
Leukocytes	Type 3 <i>leukocyte adhesion deficiency</i> (LAD3)	FERMT3 on 11q13.1 that leads to inability to provide "inside-out" signaling through $\beta$ integrins	Repeated bacterial and fungal infections, intestinal or perianal fistulae, delayed wound healing, bleeding, and high leukocytosis
Complement	<i>C3 deficiency</i>	C3 on 19p13.3	Recurrent pyogenic infections, susceptibility to <i>Neisseria meningitidis</i> and other Gram-negative bacteria
Complement	<i>Hereditary angioedema</i> (C1 inhibitor deficiency)	C1NH on 11q12.1	Recurrent episodes of angioedema at any age

*Minor primary immunodeficiencies* are relatively benign and not life-threatening. At birth, a baby has 100% of maternal IgG. The maternal antibodies degrade between the third and fifth months during a period called physiological hypogammaglobulinemia. By the sixth month most babies have already synthesized about 1/3 of their self IgG, and then they progressively save more. In some cases, the IgG synthesis is retarded up to 4–6 years to develop *transient hypogammaglobulinemia of infancy*. Gene mutations are unknown. Such babies and toddlers may suffer from recurrent infections including severe abscesses and must need a proper therapy, which includes antibiotics, immune enhancement medications, and sometimes surgical manipulations and operations. Meanwhile, the transient hypogammaglobulinemia of infancy is benign and finally results in recovery when those children are at the age of 6 years.

A decrease in IgA synthesis based on an inherited inability to secrete IgA is termed *selective IgA deficiency*. The mutations are located in the IGAD1 locus on 6p21 and the IGAD2 locus on 17p11. This benign form of primary immunodeficiency may be revealed in patients very often (about 1% of the population) but may or not have any essential clinical significance. However, IgA deficiency is occasionally linked with autoimmune pathology.

*Primary defects of interferons* may sometimes be benign. Mutations in IFN $\alpha$ 2 and IFN $\beta$ 1 genes (9p21.3) are linked with increased susceptibility to viral infections of the respiratory tract and a predisposition to some types of cancer.

**From a clinical point of view,** all medical evaluations of primary compromised patients begin with a history and physical examination. In the history it is especially important to document:

- History of present disease (general complaints, age at which infections occurred, type and sites of infectious agents, prior immunizations (and complications if any), which therapy was effective, etc.)
- Family history (in particular, early deaths of male babies)
- Past medical history (associated diseases)
- Social and occupational (for adults) history

In the physical examination, look particularly for:

- Vital signs (pulse, respiratory rate, blood pressure)
- Morphologic stigmata (e.g., syndactyly, hernias, epispadia, cryptorchidism, etc.)
- Sites of recurrent infection
- Dysbiosis in barrier organs
- Swollen lymph nodes
- Hepatosplenomegaly
- Rashes
- Telangiectasias
- Neurological disorders

Treatment for primary immunodeficiencies depends on the form of pathology and may include transplantation of the bone marrow or immunoglobulin replacement therapy in the form of lifelong intravenous immunoglobulin (IVIG) administration.

*Secondary immunocompromised conditions* can result from *HIV* infection, malnutrition, post-traumatic stress disorder, aging and immunosenescence, radiation therapy, particular medications (e.g., immunosuppressive drugs after graft transplantation, disease-modifying antirheumatic drugs, chemotherapy in malignancies, prolonged corticosteroid therapy, etc.), many types of cancer (leukemias, lymphomas, etc.), protein-losing enteropathy, burns, uremia, loss of lymphoid organs (e.g., splenectomy, appendectomy, resection of the small intestine containing Peyer's patches, etc.), and some autoimmune diseases and other disorders. Interestingly, the high-performance sports are at risk of occurrence of the secondary immunocompromised condition.

A dominant approach to treatment for secondary immunocompromised conditions is immune enhancement therapy.

### ■ Quiz

Reading a question, please choose only one right answer.

#### ❓ Question 1

X-linked agammaglobulinemia (Bruton's syndrome) is caused by gene mutation of:

1. ICOS.
2. CD19.
3. WASP.
4. BTK.

**? Question 2**

Ataxia-telangiectasia is:

1. Louis-Bar syndrome.
2. Wiskott-Aldrich syndrome.
3. Bruton's syndrome.
4. Severe combined immunodeficiency (SCID).

**? Question 3**

A gene mutation which leads to common variable immunodeficiency (CVID) is:

1. CD20.
2. BTK.
3. ATM.
4. CD132.

**? Question 4**

Another gene mutation which results in common variable immunodeficiency (CVID) is:

1. CD81.
2. WASP.
3. C3.
4. WASP.

**? Question 5**

X-linked chronic granulomatous disease is related to primary immunodeficiency of:

1. Complement.
2. Phagocytosis.
3. T cells.
4. B cells.

**? Question 6**

X-linked chronic granulomatous disease occurs in:

1. Female babies and toddlers.
2. Female and male babies and toddlers.
3. Male babies and toddlers.
4. Adult females only.

**? Question 7**

C3 deficiency leads to recurrent pyogenic infections mainly caused by:

1. *Treponema pallidum*.
2. *Helicobacter pylori*.
3. *Neisseria meningitidis*.
4. *Chlamydia trachomatis*.

**? Question 8**

The main therapy for primary B-cell immunodeficiencies is:

1. Intravenous immunoglobulin (IVIG) administration.
2. Antibiotics.

3. Bone marrow transplantation.
4. Immune enhancement therapy.

**?** Question 9

The main therapy for primary T-cell immunodeficiencies is:

1. Intravenous immunoglobulin (IVIG) administration.
2. Thymus transplantation.
3. Bone marrow transplantation.
4. Antibiotics.

**?** Question 10

The main treatment for secondary immunocompromised conditions is:

1. Bone marrow transplantation.
2. Immune enhancement therapy.
3. Antibiotics.
4. Intravenous immunoglobulin (IVIG) administration.

6

**?** Question 11

Recurrent infections are the major feature of immunodeficiencies:

1. Unknown.
2. No way.
3. Yes.
4. Probably.

**?** Question 12

*HIV/AIDS* is:

1. A secondary immunocompromised condition.
2. An autoimmune disease.
3. An allergic disease.
4. A primary immunodeficiency.

**?** Question 13

Immunodeficiency may result in cancer:

1. No.
2. In research only.
3. Yes.
4. Unknown.

**?** Question 14

Gene mutations may be revealed by:

1. ELISA.
2. Northern blot.
3. Western blot.
4. Tests on phagocytosis.

**? Question 15**

Immunosuppressive therapy may result in:

1. Secondary immunocompromised conditions.
2. HIV/AIDS.
3. Graft rejection.
4. Primary immunodeficiencies.

**? Question 16**

Zap70 gene mutation leads to:

1. Bruton's syndrome.
2. X-linked chronic granulomatous disease.
3. Immunodeficiency 48 (deficiency of CD8).
4. Deficiency of C3.

### 6.3 HIV/AIDS

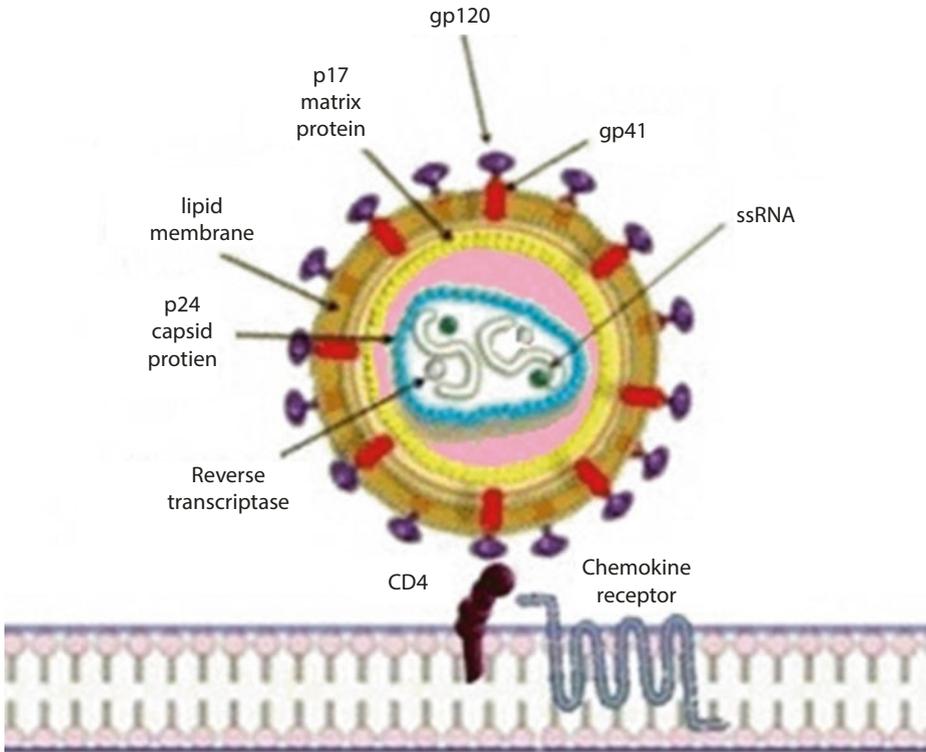
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*Human immunodeficiency virus (HIV)-1* and *HIV-2* were discovered by 2008 Nobel Laureates L.A. Montagnier and F. Barré-Sinoussi, as well as R.C. Gallo.

*HIV* is transmitted through unprotected sexual intercourse, contaminated blood transfusion, needle-sharing injections, and from mother to child during pregnancy, delivery, and breastfeeding. The key populations susceptible to *HIV* infection are (1) sex workers, (2) people who inject drugs through needle-sharing syringes, (3) men who have sex with men, (4) transgender people, (5) people who do not use condoms at high-risk sex, and (6) uncircumcised males. Currently, *HIV* is spread worldwide, but the group M *HIV-1*, in particular, is responsible for the *HIV* pandemic.

*HIV* affects type 1 CD4+ helper T cells and macrophages and progressively causes the disarmament of the immune system and loss of ability to adaptive immune responses. When CD4+ T cells decrease in the number lower than 200 cells/ $\mu$ L, clinical signs such as uncontrolled and uncommon infections, tumor growth, weight loss, etc. will commence. This condition is called acquired immunodeficiency syndrome (AIDS).

*HIV* is related to ssRNA genomic retroviruses containing reverse transcriptase; two envelope glycoproteins, gp120 and gp41; matrix protein p17; capsid protein p24; bilayer lipid membrane; and lots of other proteins (see ■ Fig. 6.1). The additional enzymes required for the development of virions are protease R, RNase H, and integrase. Envelope glycoproteins help *HIV* to anchor with a CD4+ molecule and fuse the viral particle with the host cell membrane. During the attachment, two coreceptors of T cells, CCR5 or CXCR4, take part in the process. Capsid protein p24 enables viral RNA to enter the host nucleus and the reverse transcription. The reverse transcriptase converts the ssRNA molecule into dsDNA. However, *HIV* may remain in two forms, an intact viral particle with ssRNA and integrated in the host genome virus with dsDNA (proviral DNA). The proviral DNA exploits the host cell for the transcription and translation to make new virions. The matrix protein p17 helps *HIV* in the replication and entry of the envelope in a new virion.



■ Fig. 6.1 HIV attachment to CD4+ T cell

*HIV* infection is a slow infection. WHO recommended *HIV/AIDS* staging according to clinical signs if *HIV* infection is confirmed (see ■ Table 6.2).

**From a clinical point of view,** WHO recommendations emphasize that male circumcision should be considered an efficacious intervention for *HIV* prevention in countries with high *HIV* prevalence and low male circumcision prevalence. The evidence-based study indicates that male circumcision reduces the risk of men heterosexually acquiring *HIV* infection by about 60%.

Effective *HIV* vaccine is not manufactured yet. Up-to-date approaches to chemo treatment for *HIV/AIDS* patients must be in a combination manner. There are six distinct classes of antiretroviral medicines:

- Nucleoside reverse transcriptase inhibitors
- Non-nucleoside reverse transcriptase inhibitors
- Protease inhibitors
- Integrase inhibitors
- Fusion inhibitors
- Entry inhibitors

Highly active combination antiretroviral therapy has led nowadays to a decrease in morbidity and mortality linked to *HIV/AIDS*. On the other hand, there is a problem of the drug resistance of *HIV*.

■ **Table 6.2** HIV/AIDS stages

Clinical stage	Clinical conditions
Primary HIV infection	Asymptomatic Acute retroviral syndrome
Clinical stage 1	Asymptomatic Persistent generalized lymphadenopathy
Clinical stage 2	Moderate unexplained weight loss (<10% of presumed or measured body weight) Recurrent respiratory infections (sinusitis, tonsillitis, otitis media, and pharyngitis) <i>Herpes zoster virus (HZV)</i> infection Angular cheilitis Recurrent oral ulceration Papular pruritic eruptions Seborrheic dermatitis Fungal nail infections
Clinical stage 3	Unexplained severe weight loss (>10% of presumed or measured body weight) Unexplained chronic diarrhea for >1 month Unexplained persistent fever for >1 month (>37.6 °C, intermittent or constant) Persistent oral <i>Candida albicans</i> infection (thrush)
	Oral hairy leukoplakia ( <i>Epstein-Barr virus, EBV</i> ) Pulmonary tuberculosis (current) Severe presumed bacterial infections (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia) Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis Unexplained anemia (hemoglobin <8 g/dL) Neutropenia (neutrophils <500 cells/μL) Chronic thrombocytopenia (platelets <50,000 cells/μL)
Clinical stage 4	Pneumonia caused by <i>Pneumocystis carinii</i> Recurrent severe bacterial pneumonia Chronic <i>herpes simplex virus (HSV)</i> infection (orolabial, genital, or anorectal site for >1 month or visceral herpes at any site) <i>Candida albicans</i> infection of the esophagus, trachea, bronchi, or lungs Extrapulmonary tuberculosis Kaposi's sarcoma ( <i>type 8 Human herpesvirus, HHV-8</i> ) <i>Cytomegalovirus</i> infection (retinitis or infection of other organs) Central nervous system of <i>Toxoplasma gondii</i> HIV encephalopathy <i>Cryptococcus</i> 's, extrapulmonary (including meningitis) Disseminated nontuberculous mycobacterial infection ( <i>M. bovis</i> , etc.) Progressive multifocal leukoencephalopathy Chronic cryptosporidiosis (with diarrhea) Chronic isosporiasis caused by <i>Isospora belli</i> Disseminated mycosis (e.g., histoplasmosis, coccidioidomycosis, penicilliosis) Recurrent nontyphoidal <i>Salmonella</i> bacteremia Lymphoma (cerebral or B-cell non-Hodgkin) Invasive cervical carcinoma Atypical disseminated leishmaniasis Symptomatic HIV-associated nephropathy Symptomatic HIV-associated cardiomyopathy Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)

**■ Quiz**

Reading a question, please choose only one right answer.

**? Question 1**

*HIV* is:

1. dsRNA reovirus.
2. dsDNA herpesvirus.
3. ssDNA circovirus.
4. ssRNA retrovirus.

**? Question 2**

In *HIV/AIDS*, clinical signs are starting out if a number of CD4+ T cells decrease in:

1. 50 cells/ $\mu\text{L}$ .
2. 200 cells/ $\mu\text{L}$ .
3. 1000 cells/ $\mu\text{L}$ .
4. 700 cells/ $\mu\text{L}$ .

**? Question 3**

Envelope glycoproteins help *HIV* to bind with:

1. CD8+ molecule.
2. CD19+ molecule.
3. CD3+ molecule.
4. CD4+ molecule.

**? Question 4**

Coreceptors for CD4+ molecule are:

1. CCR3 and CCR4.
2. CCR1 and CCR3.
3. CCR5 and CXCR4.
4. CCR7 and CXCR1.

**? Question 5**

*HIV* infection is:

1. An opportunistic infection.
2. A slow infection.
3. An acute infection.
4. A prion infection.

**? Question 6**

*HIV* selectively infects:

1. B cells and neutrophils.
2. NK cells and ILCs.
3. CD4+ T cells and macrophages.
4. CD8+ T cells and mast cells.

**? Question 7**

*HIV/AIDS* is:

1. A primary immunodeficiency.
2. An autoimmune disease.
3. A secondary immunocompromised condition.
4. An autoinflammatory disease.

**? Question 8**

*HIV*'s envelope proteins are:

1. gp41 and gp120.
2. L1, L2, E6, and E7.
3. gB and gN.
4. HA and NA.

**? Question 9**

Effective *HIV* vaccine is available and widespread:

1. Yes.
2. In development only.
3. Unknown.
4. Will not be ever manufactured.

**? Question 10**

Is circumcision considered an efficacious intervention for the prevention of *HIV* infection?

1. Unknown.
2. Yes.
3. Among men who have sex with men only.
4. No way.

**? Question 11**

Evidence-based study indicates that male circumcision reduces the risk of men heterosexually acquiring *HIV* infection by:

1. About 100%.
2. About 10%.
3. About 60%.
4. Among men who have sex with men only.

**? Question 12**

The modern treatment for *HIV/AIDS* patients must be in a combination manner:

1. No.
2. Unknown.
3. Among men who have sex with men only.
4. Yes.

**?** Question 13

Transgender people are related to the key populations susceptible to *HIV* infection:

1. Unknown.
2. In research.
3. Yes.
4. No.

**?** Question 14

The main approach to *HIV/AIDS* therapy is:

1. Immune enhancement therapy.
2. Chemotherapy.
3. Antibiotics.
4. Intravenous immunoglobulin (IVIg) administration.

**?** Question 15

Capsule protein p24 enables:

1. The viral RNA to enter the host nucleus.
2. The envelope to enter a new virion.
3. The ssRNA molecule to convert into dsDNA.
4. To anchor with a CD4+ molecule.

**?** Question 16

The reverse transcriptase enables:

1. The ssRNA molecule to convert into dsDNA.
2. The envelope to enter a new virion.
3. Viral RNA to enter the host nucleus.
4. To anchor with a CD4+ molecule.

## 6.4 Allergic Disorders

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**Definitions**

*Allergy* is a form of immunopathology in which adaptive immune responses are directed to allergens and are not protective. Such immune response is called **sensitization**.

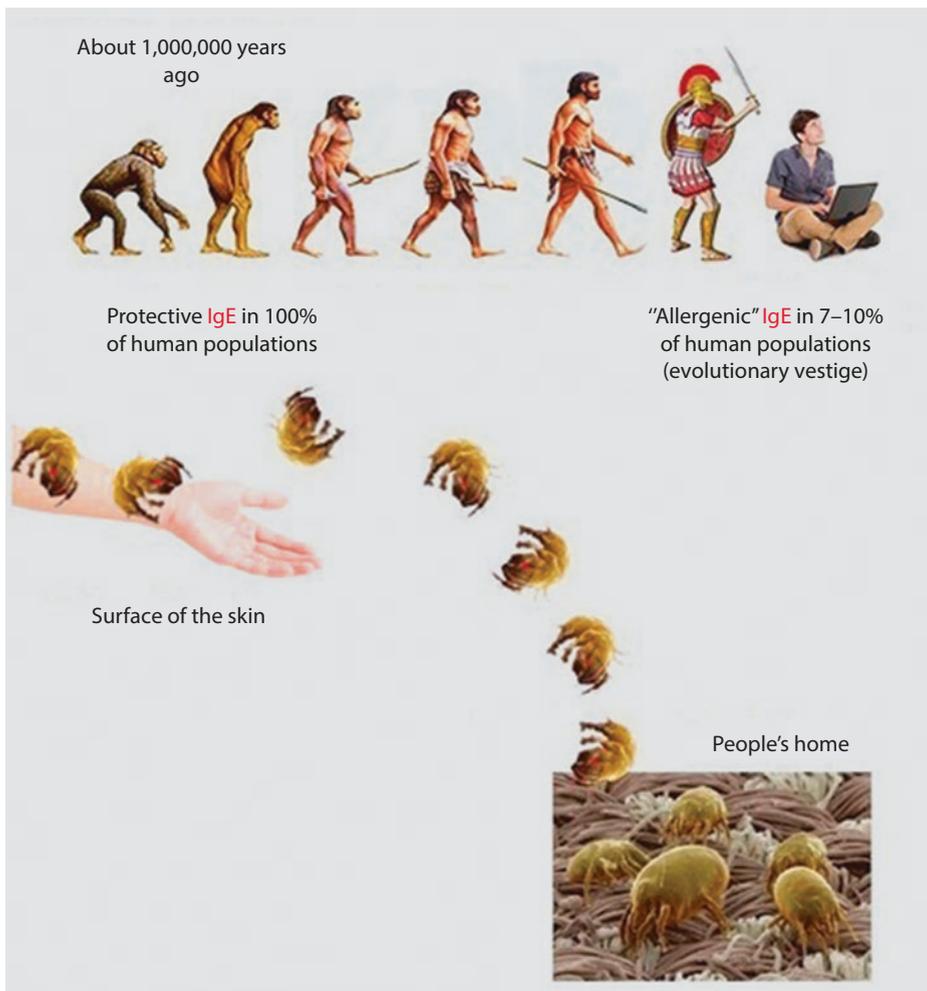
*Atopy* is the most common form of allergy based on type I hypersensitivity.

Hypersensitivity is an array of undesirable harmful reactions produced by the healthy immune system including allergies and autoimmunity. There are four types of hypersensitivity in the Gell-Coombs classification.

### 6.4.1 Type I

Type I, immediate hypersensitivity, or atopy, just occurs in selected populations of *Homo sapiens*. It is a polygenously inherited condition. The term “atopy” is used by allergists and scientists for any hyper IgE-mediated reaction induced B-cell-mediated Th2-dependent response to various allergens such as household dust, house dust mites, animal hair and skin scales, pollens, flour, food proteins, insect venoms, molds, latex, penicillin, etc. There are oligomeric components of allergen molecules, allergen-associated molecular patterns (AAMPs), which may be responsible for effective cross-linking of allergen by BCR/IgE.

The atopy appears to show a strong hereditary component as a consequence of evolution vestige (see ■ Fig. 6.2). From an evolutionary point of view, house dust mites,



■ Fig. 6.2 The condition of atopy as an evolutionary vestige

*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, are the “kings of allergens” or panallergens. It is probable that they used to be skin parasites in ancient humans in the Stone Age.

Exposure to allergens may be by inhalation, ingestion, injection, or direct contact. In the course of B-cell-mediated immune response, plasma cells are stimulated by type 2 helper CD4+ T cells to produce IgE antibodies specific to one allergen or allergen group. The difference between a common B-cell-mediated response and a type I hypersensitivity response is that in type I hypersensitivity, the IgE antibodies predominate instead of IgM, IgG, or IgA immunoglobulins. The IgE antibodies bind to type I Fcε receptors (FcεRI) on the surface of mast cells and circulating basophils. After exposure to the same allergen, the allergen cross-links the bound IgE on target cells that leads to degranulation and the secretion of inflammatory mediators.

## 6

Type I hypersensitivity reactions may commonly be divided into two phases, the early phase reaction and the late phase reaction. The **early phase** typically occurs within 10–20 minutes, or even seconds, following allergen exposure, caused by the release of preformed mediators such as histamine, serotonin, chemotactic peptides for neutrophils and eosinophils, enzymes, etc. These mediators affect the nerve cells causing an itch, smooth muscle contraction (e.g., asthmatic attack), mucus production by goblet cells, increase in capillary permeability and subsequent tissue edema, and recruitment of neutrophils and eosinophils. The **late phase** develops over 6–12 hours and is mediated by newly formed mediators such as thromboxane, leukotriene C4 (LTC4), leukotriene B4 (LTB4), prostaglandin D2 (PGD2), platelet-activating factor (PAF), cytokines, and chemokines that act on surrounded tissues enhancing the inflammatory process. Endothelial cells express those adhesion molecules (L-selectins, PSGL-1, ICAMs, LFA-1, and PECAM-1), which facilitate the recruitment and activation of neutrophils, eosinophils, and lymphocytes from the blood into the site of the allergic inflammation. Commonly, the infiltrating cells contain a high proportion of eosinophils. The eosinophils release a variety of inflammatory molecules including major basic protein, eosinophilic peroxidase, IL5, etc. The involved Th2 cells secrete IL4, IL5, IL6, IL13, etc. and affect plasma cells, which promote IgE isotype switching. The inflammatory process becomes long term.

Type I hypersensitivity is responsible for atopic dermatitis, perennial and seasonal allergic rhinitis, bronchial asthma, food allergies, insect allergies, anaphylactic shock, etc. Allergic skin tests and investigation of blood IgE are very useful for diagnosing the atopic allergic conditions. Recently, a new endotype of atopy has been identified, the *entopy*, which includes local allergic rhinitis, local allergic conjunctivitis, and local allergic asthma.

### 6.4.2 Type II

Type II hypersensitivity, or cytotoxic reaction, is based on an advanced B-cell-mediated response when antibodies are bound to antigens, which are present on the body's self-cells. The antigens may also be self-antigens (e.g., agglutinogens of erythrocytes, which define groups of the human blood, Rh antigen, etc.). The extrinsic antigens (e.g., infectious pathogens or drugs) are adsorbed onto the cells upon exposure to them during

infection or treatment. IgG and IgM antibodies bind to these antigens to constitute complexes, which activate the complement via the classical pathway to lyse cells with the antigens.

Type II hypersensitivity is responsible for drug leukopenia, hemolytic anemia, thrombocytopenia, Goodpasture's syndrome, myasthenia gravis, pemphigus vulgaris, and hyperacute graft rejection. Cytopenic tests may be useful for the diagnostic of conditions associated with type II hypersensitivity.

### 6.4.3 Type III

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Type III hypersensitivity is based on an advanced B-cell-mediated immune response when the formed immune complexes are not removed and induce an inflammatory reaction in the blood vessels in various tissues, called vasculitis, which may result in immune complex diseases.

Medium-sized complexes, formed in the obvious excess of antigen, C3b-free, are the highest pathogenic immune complexes because they cannot be eliminated and must be easily deposited in the tissues. Small complexes, constituted in the slight excess of antigen, contain C3b and may be phagocytosed but also bound to the sites of deposition in the vessels. Induced by deposition of the immune complexes, inflammation involves complement, phagocytes, mast cells, enzymes, cytokines, and chemokines and exerts a destructive potency. Arthus reaction, a typical skin condition on the base of type III hypersensitivity, is characterized by local erythema and induration due to skin vasculitis, which develops within several hours and/or days after second exposure to antigen.

Type III hypersensitivity is responsible for serum sickness, exogenous allergic alveolitis, post-streptococcal glomerulonephritis, reactive arthritis, Henoch-Schönlein purpura, rheumatoid arthritis, systemic lupus erythematosus (SLE), and chronic graft vasculopathy. Immunofluorescence microscopy can be used to visualize the immune complexes in the tissues.

### 6.4.4 Type IV

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Type IV, delayed hypersensitivity, is based on an inflammatory CD4+ T-cell-mediated immune response. Under different circumstances and the presence of different inducers, this form of immune response may be either beneficial or harmful. In any case, the response to the venom of *Poison oak* (see ■ Fig. 6.3) is always detrimental. A variety of the harmful inducers may be home chemicals, glue, gold, nickel, latex, etc. In the beginning, type 1 helper CD4+ T cells (Th1) are activated by an antigen-presenting cell (macrophage). When the antigen is exposed again, the memory CD4+ T cells will activate macrophages, making them release pro-inflammatory cytokines, hydrolytic enzymes, and nitric oxide; transform into multinucleated giant cells; recruit other cells; and cause for 48–72 hours immune inflammation with potential damage to tissues and growth of granulomas. The typical skin sample of type IV hypersensitivity is the induration and erythema around the injection site of tuberculin, which takes place in the Mantoux test.

■ Fig. 6.3 Poison oak



Type IV (delayed) hypersensitivity is responsible for allergic contact dermatitis; tissue damage in a variety of infectious diseases like tuberculosis, leprosy, syphilis, and molds; chronic graft rejection; and some autoimmune disorders such as multiple sclerosis, autoimmune myocarditis, Hashimoto's thyroiditis, type 1 diabetes mellitus, and celiac disease. In vitro lymphoblast transformation test (LTT) and in vivo patch tests help identify which allergens cause the sensitization.

**From a clinical point of view,** any allergist should define the *atopy* in patients with different allergic disorders very precisely. The *atopy* is the “queen” of all types of allergy and pseudo-allergy. In predisposed atopic individuals, the term *atopic march* means a subsequent change of target organs in the following order: the skin, nose/conjunctives, and bronchi. Some elder patients may simultaneously develop all atopic conditions. Also, any allergist should be familiar with that only in atopic individuals the allergen-specific immunotherapy (ASIT) may be used as a high-effective approach.

All medical evaluations of patients suffering from allergy begin with a history and physical examination.

In the history, it is especially important to document:

- History of present disease (general complaints, type of onset and signs, which factors seem to be provocative, link with a certain period of the year, which therapy seems to be effective, etc.)
- Family history (in particular, atopic heredity)
- Past medical history (age at which first allergic signs occurred, type and sites of allergic signs, which therapy was effective, associated diseases, etc.)
- Social and occupational (in adults) history

In the physical examination, look particularly for:

- Vital signs (pulse, respiratory rate, blood pressure)
- State of the skin (which morphological lesions and their sites, itching, secondary infections, etc.)
- Presence of edema and redness of the conjunctives

- Nasal obstruction, type and values of discharge and sputum
- Wheezing, dry rale, expiratory dyspnea

*Atopic dermatitis* is a chronic itchy skin inflammatory disease based on type I hypersensitivity and mutations in the filaggrin gene on 1q21.3. In predisposed persons, or atopic individuals, house dust mite allergens and other allergens are inhaled, ingested and enter the skin (so-called epicutaneous sensitization), and trigger advanced B-cell-mediated immune response to the predominant formation of IgE antibodies, which are carried to the skin to be bound to FcεR1s on mast cells. Repeated exposure to these allergens results in chronic skin inflammation with many types of skin lesions and severe itching that can have a significant impact on the quality of life for patients in particular if the skin lesions are extensive. As a rule, first symptoms of atopic dermatitis occur at the early age in babies and toddlers and may next appear as recurrences throughout the life. In some cases, the immunologic tolerance to causative allergens is restoring to lead to the long-term remission of the disease. However, some patients may develop eczema; secondary bacterial, fungal, and viral infections; and rough lichenification as complications of the main allergic inflammatory process.

Treatment for the disease includes emollients, corticosteroid gels, creams and ointments, antihistamines, antimicrobial medications if required, and allergen-specific immunotherapy (ASIT).

*Food allergy* is an allergic syndrome linked to IgE-dependent hypersensitivity to food proteins, in particular those derived from milk products, seafood, nuts, mushrooms, flour, eggs, fruit, vegetables, etc. In some cases, pseudo-allergic (i.e., immune independent) mechanisms contribute to the food allergy. Patients of this polymorphic condition may develop isolated itching, dermatitis, hives, edema, and even anaphylactic shock. Having the immature maintenance mechanisms of natural tolerance to food proteins, children at the early age suffer from food allergy much more often than grown-up persons who develop food allergic reactions in 0.5% cases only. The effective prevention of food allergy in adults is avoidance measures concerning causative food allergens throughout the life, whereas the approaches to oral ASIT are still in progress.

*Allergic rhinitis (rhinoconjunctivitis)* may be developed by atopic individuals in two forms: (1) perennial and (2) seasonal rhinitis (rhinoconjunctivitis). Patients complain of frequent or constant episodes of nasal obstruction, watery discharge, sneezing, loss of smell, conjunctival swelling and redness, and postnasal cough. The perennial rhinitis commonly begins at the age of 3–5 years and manifests itself as some of these symptoms all year round and sometimes throughout the life being severe, persistent, and complicated by nasal polyps, sinusitis, and asthma or conversely mild and subtle. Allergens of house dust mites, cats, dogs, and other pets and molds are the main responsible factors for IgE-dependent hypersensitivity in patients of perennial rhinitis. The seasonal rhinitis or “pollinosis” may begin at any age across large areas of both hemispheres during certain periods of the year when gramineous plants and trees pollinate since the sensitization is caused by allergenic pollens of birch, hazel tree, oak, fescue, ryegrass, timothy, ragweed, artemisia, and a wide variety of plants.

Treatment for the disease consists of corticosteroid nasal sprays, antihistamines, anti-leukotrienes, and allergen-specific immunotherapy (ASIT).

*Asthma* is caused by the same allergens as allergic rhinitis in atopic individuals, pathogenically linked to chronic “eosinophil inflammation,” Th2 polarization, bronchial spastic episodes, edema, and hypersecretion, and clinically manifests itself as wheezing and coughing. As a rule, asthma may begin at the age of 4 years and older having a significant long-term impact on the quality of life for patients. There is also described the asthma subgroup, preferentially adult-onset asthma, associated with “neutrophil inflammation” and IL8 (CXCL8), IL17, TNF $\alpha$ , etc. and resembling, but not identical, with *chronic obstructive pulmonary disease (COPD)*. Asthmatic attacks are able to be triggered not only by causative allergens but by respiratory infection, exercise, cold air, drugs like aspirin or pollutants like sulfur dioxide, phthalates, etc. *Tiffeneau index* displays the ratio between the first second forced expiration (FEV1) and full vital capacity (FVC), accounts for normal values of about 80%, and diminishes in cases of the bronchial obstruction. Airway irritability may be also measured by an inhaled cholinergic agent such as methacholine taking into consideration a decrease of the airway flow in 20% and more.

Treatment for the disease includes inhaled corticosteroids, antihistamines, anti-leukotrienes, anti-IgE and anti-cytokine-based monoclonal antibodies,  $\beta$  agonists if required, and allergen-specific immunotherapy (ASIT).

#### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

Type III hypersensitivity is caused by:

1. Large immune complexes.
2. Macrophages activated by CD4+ T cells.
3. Medium-sized immune complexes.
4. Elevated concentration of IgE.

#### ? Question 2

Type I hypersensitivity early phase is characterized by:

1. Release of histamine.
2. Release of pro-inflammatory cytokines.
3. Reactive oxygen species (ROS).
4. Increase in leukotrienes.

#### ? Question 3

Type I hypersensitivity is linked with:

1. Large immune complexes.
2. Macrophages activated by CD4+ T cells.
3. Medium-sized immune complexes.
4. Elevated concentration of IgE.

#### ? Question 4

Type I hypersensitivity late phase is characterized by:

1. Release of pro-inflammatory cytokines.
2. Release of histamine.

3. Reactive oxygen species (ROS).
4. Increase in leukotrienes.

**?** Question 5

Seasonal allergic rhinitis refers to:

1. Type IV hypersensitivity.
2. Type II hypersensitivity.
3. Type I hypersensitivity.
4. Type III hypersensitivity.

**?** Question 6

Type 2 helper T cells upregulate:

1. Type III hypersensitivity.
2. Type II hypersensitivity.
3. Type I hypersensitivity.
4. Type IV hypersensitivity.

**?** Question 7

Type 1 helper T cells upregulate:

1. Type IV hypersensitivity.
2. Type I hypersensitivity.
3. Type II hypersensitivity.
4. Type III hypersensitivity.

**?** Question 8

The keystone cytokine of type 2 helper T cells is:

1. IL1.
2. IL4.
3. IL35.
4. IFN $\gamma$ .

**?** Question 9

Activation of eosinophils may be caused by:

1. IL8.
2. IL1.
3. IL5.
4. TGF $\beta$ .

**?** Question 10

Allergic contact dermatitis is related to:

1. Type IV hypersensitivity.
2. Type I hypersensitivity.
3. Type II hypersensitivity.
4. Type III hypersensitivity.

**? Question 11**

Atopic dermatitis refers to:

1. Type III hypersensitivity.
2. Type II hypersensitivity.
3. Type I hypersensitivity.
4. Type IV hypersensitivity.

**? Question 12**

The keystone cytokine of type 1 helper T cells is:

1. IFN $\gamma$ .
2. IL4.
3. IL17.
4. M-CSF.

**? Question 13**

Type IV hypersensitivity is caused by:

1. Large immune complexes.
2. Macrophages activated by CD4+ T cells.
3. Medium-sized immune complexes.
4. Increased concentration of IgE.

**? Question 14**

Atopic bronchial asthma is related to:

1. Type III hypersensitivity.
2. Type II hypersensitivity.
3. Type I hypersensitivity.
4. Type IV hypersensitivity.

**? Question 15**

Type IV hypersensitivity may be assessed in:

1. ELISA.
2. LTT.
3. Cytotoxicity assay.
4. Western blot.

**? Question 16**

Response to the venom of *Poison oak* leads to:

1. Type III hypersensitivity.
2. Type I hypersensitivity.
3. Type II hypersensitivity.
4. Type IV hypersensitivity.

## 6.5 Autoimmune and Autoinflammatory Disorders

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### Definitions

*Autoimmune diseases* are caused by the breakdown in natural tolerance to self-antigens and characterized by polymorphic clinical symptoms and commonly progressive course of the disorders.

*Autoinflammatory diseases* are a group of disorders caused by the excessive mechanisms of innate immunity without the involvement of adaptive immune responses and immune memory.

*Autoimmune diseases* are a group of disorders characterized by the breakdown in self-tolerance to a wide variety of autoantigens. In genetically predisposed persons, these diseases occur as a multistep process in which environmental factors including infections have key roles in the development of abnormal innate and adaptive immune responses. Normally, self-reactive T cells and B cells are either eliminated through apoptosis before entering circulation and functioning within the immune system, placed into a state of anergy, or inactivated by immunoregulatory mechanisms. When maintenance of self-tolerance fails, self-reactive lymphocytes and autoantibodies attack the body's self-tissues.

*Mutations* may be crucial in the pathogenesis of autoimmune conditions. Some autoimmune diseases and syndromes are monogenic, whereas the most disorders exert polygenic inheritance. Mutation in a single AIRE gene (21q22.3) leads to a rare autosomal recessive disease, *autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED)*, which is characterized by a wide heterogeneous phenotype though. The AIRE gene encodes a transcription regulator important for the negative selection of thymocytes in the thymus.

Mutations in FAS gene (10q23.31) and FASLG gene (1q24.3) result in *autoimmune lymphoproliferative syndrome (ALPS)*. ALPS is categorized by nonmalignant lymphadenopathy and splenomegaly and cytopenias. It is known these genes are involved in apoptosis, a tool for the negative selection in the course of T-cell and B-cell lymphopoieses.

Mutations in a single FOXP3 gene (Xp11.23) lead to *x-linked immunodysregulation, polyendocrinopathy, enteropathy (IPEX) syndrome*, a recessive condition, which is characterized by both systemic autoimmune and primary immunodeficiency features. The FOXP3 gene encodes a key transcription factor for the development of nTreg cells.

In the course of adaptive responses, random mutations of BCRs and probably TCRs may occur *ex novo*, and in an casual manner these novel antigen-recognizing receptors may sense autoantigens.

Moreover, there are T cells, which have simultaneously *two different TCRs*, and how two TCRs on one T cell influence each other is known little. It is possible if such TCRs on a single T cell are directed to both an exogenous antigen and endogenous antigen; T-cell-mediated adaptive response to the exogenous antigen may lead to affect autoantigen and induce autoimmune disorder.

However, some additional mechanisms are necessary so that an autoimmune disease might start.

1. The concept of *molecular mimicry* describes a situation in which a foreign antigen can initiate immune responses when T cells or B cells cross-recognize the “self.” Some infections, such as *group A streptococci*, have antigens that are similar (but not identical) to the heart’s and kidney’s self-antigens. The cross-reactive immune responses to the heart and kidney proteins may lead to autoimmune disorders in these organs.
2. Self-antigens of *immunoprivileged organs* such as the anterior chamber of the eye are present in the blood at very low concentrations and result in clonal ignorance. Upon trauma of one eye, the self-antigens enter the blood in large quantity and induce autoimmunity, in particular, sympathetic ophthalmia, or bilateral diffuse granulomatous uveitis.
3. Some viruses and bacteria can produce *superantigens*, which are polyclonal activators of T cells, up to 20% of the body’s T cells during an infectious episode. Simultaneously, the immune system provides the process with massive cytokine release. Among the T cells, there are self-reactive T cells in the state of anergy that may be activated cause autoimmunity. Such a mechanism is described for such autoimmune diseases as *type 1 diabetes mellitus*, *rheumatoid arthritis*, and *Kawasaki disease*.

6

The inflammatory process in any autoimmune diseases may be based on the mechanisms of type II, type III, or type IV hypersensitivities.

You can see a list of autoantibodies that can be used as diagnostic serologic markers for autoimmune diseases in ■ Table 6.3.

The role of autoantibodies as diagnostic serologic markers for autoimmune diseases appears to be limited due to their not insufficient sensitivity. Treatment for autoimmune diseases depends on the type and severity of the disorder. Nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and various immunosuppressive monoclonal antibodies are often employed.

**From a clinical point of view,** therapy for autoimmune and autoinflammatory diseases usually improves symptoms but does not typically cure these diseases. The suppression of inflammatory genes in immune cells and restoration to self-tolerance may be a promising therapeutic approach in the near future.

*Autoinflammatory diseases* are a relatively new group of diseases that are distinct from autoimmune diseases. However, autoimmune and autoinflammatory diseases share common characteristics in that both groups of disorders, which are attacked by the immune system concerning their self-tissues and they both lead to the increased inflammation. As opposed to autoimmune diseases, autoinflammatory conditions are caused by mechanisms of the innate immune system only without the involvement of the adaptive immune responses and immune memory.

Examples of autoinflammatory conditions are *Familial Mediterranean fever* (caused by mutation in MEFV gene (16p13.3), which encodes protein pyrin), deficiency of the IL1ra (caused by an uncontrolled amount of IL1), and neonatal-onset *multisystem inflammatory disease* (caused by mutation in NLRP3 gene (1q44), which encodes protein cryopyrin).

## 6.5 · Autoimmune and Autoinflammatory Disorders

■ **Table 6.3** Autoantibodies in autoimmune diseases

Autoantigen	Autoantibody	Autoimmune disease
Double-stranded DNA	Anti-dsDNA	Systemic lupus erythematosus (SLE)
Cyclic citrullinated peptide	Anti-CCP	Rheumatoid arthritis
Phospholipid	Antiphospholipid	Antiphospholipid syndrome
Myelin basic protein	Anti-MBP	Multiple sclerosis
A lot depending on localization	A lot depending on localization	Paraneoplastic syndromes and autoimmune encephalitis
Nicotinic acetylcholine receptor Muscle-specific receptor tyrosine kinase	Anti-AChR Anti-MuSK	Myasthenia gravis
Topoisomerase	Anti-topoisomerase	Systemic scleroderma
Centromere	Anti-centromere	Limited scleroderma (CREST syndrome)
FcεRI	Anti-FcεRI	Chronic idiopathic urticaria
Desmoglein-3	Anti-desmoglein-3	Pemphigus vulgaris
Ribonucleoproteins	Anti-La/SS-B	Primary Sjögren's syndrome
Intrinsic factor	Anti-intrinsic factor	Pernicious anemia, atrophic gastritis
Mitochondria Sp100 nuclear antigen Nucleoporin 210 kDa	Anti-mitochondrial; Anti-sp100; Anti-glycoprotein-210	Primary biliary cirrhosis
Smooth muscle proteins Soluble liver antigen/liver pancreas	Anti-smooth muscle; Anti-SLA/LP	Type I autoimmune hepatitis
Liver cytosol antigen 1 Liver/kidney microsomes	Anti-LC-1; Liver/kidney microsomal (anti-LKM-1)	Type II autoimmune hepatitis
Thyroglobulin	Anti-thyroglobulin	Hashimoto's thyroiditis
Thyrotropin receptor	Anti-TSH receptor	Graves' disease
Glutamate decarboxylase	Anti-GAD	Type 1 diabetes mellitus
Endomysium Tissue transglutaminase Deamidated gliadin peptide	Anti-endomysial Anti-tTG Anti-DGP	Celiac disease
<i>Saccharomyces cerevisiae</i>	ASCA	Crohn's disease
Atypical perinuclear antineutrophilic cytoplasmic proteins Human tropomyosin isoform 5 (in colonocytes)	Atypical pANCA Anti-hTM5	Ulcerative colitis

(continued)

**Table 6.3** (continued)

Autoantigen	Autoantibody	Autoimmune disease
Glomerular basement membrane protein Type IV collagen	Anti-GBM Anti-collagen IV	Goodpasture's syndrome
Entactin	Anti-entactin	Autoimmune glomerulonephritis
Endothelial cell antigen Neutrophilic cytoplasmic antigens Ferritin	Anti-ECA Anti-NCA Anti-ferritin	Systemic vasculitides
C1q	Anti-C1q	Hypocomplementemic urticarial vasculitis
GPIIb/IIIa (CD41/CD61) GPIbIX (CD42a and CD42b)	Anti-GPIIb/IIIa (CD41/CD61) Anti-GPIbIX (CD42a/CD42b)	Immune thrombocytopenic purpura

Interestingly, a novel condition, *cytokine storm*, characterized by massive cytokine release, is very similar to any autoinflammatory disease. Cytokine storms can occur in a variety of infectious and noninfectious diseases including *systemic inflammatory response syndrome (SIRS)*, *graft-versus-host disease (GVHD)*, *sepsis*, *influenza pandemic*, etc.

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

Breakdown of the self-tolerance results in:

1. Autoinflammatory disorders.
2. Tissue homeostasis.
3. Physiological pregnancy.
4. Autoimmune diseases.

#### ? Question 2

Clonal ignorance is:

1. Adaptive immune responses to self-antigens at a low concentration.
2. The activation of innate immunity.
3. The unresponsiveness of peripheral lymphocytes to self-antigens at a low concentration.
4. Adaptive immune responses to self-antigens at a high concentration.

#### ? Question 3

Molecular mimicry is:

1. Cross-recognition of various allergens.
2. Somatic hypermutations.

3. Resistance of bacteria to antibiotics.
4. Cross-recognition of the “self” similar to “non-self” by T cells and B cells.

**?** **Question 4**

An immunoprivileged organ is:

1. The eye.
2. The spleen.
3. Lymph nodes.
4. The skin.

**?** **Question 5**

Superantigens are:

1. Antigens uploaded on Class I HLA molecules.
2. Polyclonal activators of T cells.
3. Allergens derived from *Dermatophagoides farinae*.
4. Antigens uploaded on Class II HLA molecules.

**?** **Question 6**

Autoimmune and autoinflammatory diseases are:

1. The same.
2. Different according to the involvement of either macrophages or eosinophils.
3. Different according to the involvement of either innate immunity or both types of immunity.
4. Different according to the participation of either T cells or B cells.

**?** **Question 7**

Autoimmune disorders may be based on:

1. Types I–III hypersensitivity in most cases.
2. Types I–IV hypersensitivity in all cases.
3. Types II–IV hypersensitivity in most cases.
4. Type I hypersensitivity in all cases.

**?** **Question 8**

Autoantibodies to *Saccharomyces cerevisiae* may serve a diagnostic serologic marker for:

1. Crohn’s disease.
2. Celiac disease.
3. Ulcerative colitis.
4. Type 1 diabetes mellitus.

**?** **Question 9**

Autoantibodies to dsDNA may serve a diagnostic serologic marker for:

1. Type 1 diabetes mellitus.
2. Systemic lupus erythematosus (SLE).
3. Graves’ disease.
4. Myasthenia gravis.

**? Question 10**

Autoantibodies to Fc $\epsilon$ RI may serve a diagnostic serologic marker for:

1. Systemic scleroderma.
2. Primary biliary cirrhosis.
3. Chronic idiopathic urticaria.
4. Hashimoto's thyroiditis.

**? Question 11**

Various immunosuppressive monoclonal antibodies are often used in autoimmune diseases:

1. Yes.
2. Unknown.
3. No.
4. Never.

**? Question 12**

The modern therapy for autoimmune and autoinflammatory diseases:

1. Improves symptoms but does not typically cure these diseases.
2. Is completely effective in most cases.
3. Even does not improve symptoms.
4. Cures these diseases.

**? Question 13**

B-cell clonal anergy is characterized by:

1. Increase in CD28 expression.
2. Cross-linking antigens.
3. Clonal deletion.
4. Increase in CTLA-4 expression.

**? Question 14**

T-cell clonal anergy is characterized by:

1. Clonal deletion.
2. Increase in CD28 expression.
3. Increase in CTLA-4 expression.
4. Complement activation.

**? Question 15**

Maintenance of the self-tolerance results in:

1. Autoimmune disorders.
2. Immunological infertility.
3. Tissue homeostasis.
4. Atopic allergic diseases.

**? Question 16**

Deficiency of the IL1ra is a sample of:

1. The autoinflammatory disorder.
2. The allergic disease based on type I hypersensitivity.

3. The allergic disease based on type IV hypersensitivity.
4. The autoimmune disease.

## 6.6 Immunology of Cancer

### Definitions

*Cancer immunoediting* is the concept of progressive disarmament of the immune system with eventual tumor-promoting activity caused by cancerous process.

Actually, it is known that there is a variety of certain mechanisms and conditions recruited into the carcinogenesis including the “critical mass” of mutations; the epigenomic disorders like damage to the mitochondria in the cytoplasm; the insulin resistance, hyperinsulinemia, and high expression of insulin-like growth factor 1 (IGF-1); the protumorigenic environment in adipose tissue in obesity; the inherited defects of cell cycle and apoptosis; the viral impacts; and the breakdown of immunological surveillance. Among cancer-inducing viruses, there are *Epstein-Barr virus (EBV)* (Burkitt’s lymphoma), *human herpesvirus 8 (HHV-8)* (Kaposi’s sarcoma), *human papillomavirus (HPV)* (cervical cancer), *hepatitis B virus* (liver cancer), etc. In some cases, for a cancer-inducing virus, a specific cofactor is required, e.g., in Africa, malarial plasmodia take part along with EBV in the processes of development of Burkitt’s lymphoma in children.

To date, understanding how and why the immune system misses cancer development and progression has been one of the most challenging questions in immunology. It is probable that the immune system plays a dual role in cancer. On the one hand, it can suppress tumor growth by destroying cancer cells, and, on the other hand, certain components of the immune system may promote tumor progression either by selecting for tumor cells, which are more ready to survive, or by establishing the tumor microenvironment to facilitate cancer outgrowth. The concept of *cancer immunoediting* integrates the immune system’s dual host-protective and tumor-promoting roles. Immunoediting has three major phases: (1) elimination, (2) equilibrium, and (3) escape.

1. In the course of the *elimination phase*, both innate immunity and adaptive immune responses are involved. Cells of the innate immune system such as NK cells, NKT cells, and macrophages attack a growing tumor, promote inflammatory mediators including reactive oxygen species (ROS), and stimulate the production of IFN $\gamma$ , IL12, CXCL9 (monokine induced by IFN $\gamma$ , MIG), CXCL10 (IFN $\gamma$ -induced protein 10, IP-10), and CXCL11 (IFN-inducible T-cell  $\alpha$  chemoattractant – I-TAC). These chemokines promote tumor cells’ death by blocking the formation of new blood vessels. Activated NK cells and NKT cells trigger the apoptosis of cancer cells. At the same time, dendritic cells recognize tumor antigens and initiate T-cell-mediated responses. At the end of the elimination phase, CD8+ T cells and CD4+ T cells migrate to the tumor site, damage tumor cells, and then kill them. However, part of the cancer cells may survive.
2. In the course of the *equilibrium phase*, which may last for many years, genetically unstable and rapidly mutating tumor cells consequently acquire partial resistance

to the immune system. It is shown that the balance of IL12 promoting cancer elimination and IL23 promoting cancer persistence maintains tumors in equilibrium. Respectively, since IL23 is an upregulation of IL17 production, Th17 may play a negative role in the maintenance of equilibrium. In addition, the recognition of tumor-associated molecular patterns (TAMPs) by PRRs may lead to chronic inflammation favoring tumor progression. Also, high proportions of CD8+ T cells, NK cells, and  $\gamma\delta$ T cells and low ratios of NKT cells, nTreg cells, and myeloid-derived suppressor cells (MDSCs) are found during this phase. Importantly, tumor antigen-specific T cells can arrest tumor growth only in the presence of IFN $\gamma$  and TNF, which may induce tumor senescence. If IFN $\gamma$  and TNF decrease, the same T cells promote angiogenesis and carcinogenesis.

3. In the course of the *escape phase*, tumor cells completely resistant to the immune system start to grow and expand in an uncontrolled manner, which eventually leads to malignancies that may infiltrate the surrounding healthy tissue and metastasize. Tumor infiltration by tumor-associated macrophages (TAMs) and type 2 tumor-associated neutrophils (TAN-2) has been shown to be a poor prognosis in some tumor types, e.g., breast cancer, ovarian cancer, and lymphoma. To date, there are many unclear questions related to this phase. Concerning the immune system, pathologic tolerance takes place.

6

Cancer-specific markers are mainly proteins, which are produced by cancer cells in a large number or by healthy cells of the body in a smaller quantity in response to cancer conditions (see ■ Table 6.4). They can be revealed in the blood, tumor tissue, urine, stool, and other tissues in cancer patients and healthy persons.

Cancer-specific markers may be useful in screening tests that aim to detect cancer conditions early, but, unfortunately, they have insufficiently high sensitivity and specificity, except for PSA. To date, such markers have not been identified for every type of cancer, and cancer researchers are turning to proteomics to develop novel biomarkers, which can be exploited to determine a tumor in its early stages and to predict the effectiveness of treatment and the chance of cancer recurrence after proper treatment.

Immunotherapy is used in cancer treatment as an additional approach. There were attempts to exploit cytokines such as IL2 and IFN $\gamma$ , oncolytic viruses, monoclonal antibodies, etc. The way in which the host dendritic cells recognize and uptake tumor antigens to present to naive CD4+ and CD8+ T cells may currently be the key to success in cancer immunotherapy.

**From a clinical viewpoint,** *dendritic cell-based vaccines* are mainly used in cancer immunotherapy. In order to trigger a CD8+ T-cell-mediated immune response and other types of responses in cancer patients, dendritic cells (DCs) have to present the relevant tumor antigens. For this purpose, either the preparation of autologous tumor lysates, or Class I HLA molecules associated with tumor antigen-derived peptides, or transfection of DCs with RNA encoding a specific cancer antigen is used. At the next stage, DCs with antigens of interest should become mature. Subsequently, DCs may administer to a cancer patient via intradermal, intravenous, or intralymphatic injection.

■ **Table 6.4** Cancer-specific markers

Cancer	Serum cancer-specific marker
Liver cancer and germ cell tumors	$\alpha$ fetoprotein (AFP)
Colorectal cancer and other GI cancers	Carcinoembryonic antigen (CEA)
Gastric cancer, pancreatic cancer, gallbladder cancer, and bile duct cancer	CA19-9
Lung cancer	Cytokeratin fragment 21-1
Small-cell lung cancer and neuroblastoma	Neuron-specific enolase (NSE)
Thyroid cancer	Thyroglobulin, calcitonin
Neuroendocrine tumors	Chromogranin A (CgA)
Multiple myeloma, chronic lymphoblast leukemia	$\beta_2$ microglobulin (B2M)
Lymphoma, leukemia, germ cell tumors, melanoma, and neuroblastoma	Lactate dehydrogenase
Non-Hodgkin lymphoma	CD20
Multiple myeloma and Waldenström's macroglobulinemia	Elevated count of immunoglobulins
Breast cancer	CA15-3/CA27.29
Ovarian cancer	CA-125, human epididymis protein 4 (HE4), 5-protein signature (OVA1 <sup>®</sup> )
Prostate cancer	Prostate-specific antigen (PSA)

■ **Quiz**

Reading a question, please choose only one right answer.

❓ **Question 1**

Cancer may be caused by viruses:

1. No.
2. In research.
3. Yes.
4. Unknown.

❓ **Question 2**

Immunoediting is the concept about:

1. The changing interactions between cancer and immune system.
2. The constant immune surveillance.
3. The permanent tumor-promoting activity of the immune system.
4. The permanent immunodeficiency in cancer.

**? Question 3**

The equilibrium phase is characterized by:

1. The obvious damage to tumor cells and killing them.
2. Tumor-promoting activity of the immune system.
3. Senescence of tumor cells.
4. Balance between cancer and immune system.

**? Question 4**

The elimination phase is characterized by:

1. The balance between cancer and immune system.
2. Obvious damage to tumor cells and killing them.
3. The neutral state of the immune system regarding to cancer.
4. Tumor-promoting activity of the immune system.

**? Question 5**

The escape phase is depicted by:

1. The neutral state of the immune system regarding to cancer.
2. Complete resistance of tumor cells to immune mechanisms.
3. Senescence of tumor cells.
4. Obvious damage to tumor cells and killing them.

**? Question 6**

The recognition of tumor-associated molecular patterns (TAMPs) by PRRs may play:

1. Obvious protective role only.
2. Obvious tumor-promoting role only.
3. "Double-edged sword" role to promote cancer or protect from it.
4. Unknown.

**? Question 7**

These cells can trigger the apoptosis of cancer cells:

1. Macrophages and neutrophils.
2. CD4+ T cells and B cells.
3. NK cells, NKT cells, and CD8+ T cells.
4. Mast cells and basophils.

**? Question 8**

These chemokines can block the formation of new blood vessels for tumor cells:

1. CXCL9, CXCL10, and CXCL11.
2. CCL19, CCL21, and CXCL13.
3. CCL17, CCL25, CCL27, and CCL28.
4. CCL21, CCL25, and CXCL12.

**? Question 9**

These cells can produce reactive oxygen species (ROS):

1. CD8+ T cells.
2. Neutrophils and macrophages.

3. NK cells.
4. B cells and plasma cells.

**?** **Question 10**

Tumor infiltration by these cells has been shown to be a poor prognosis in some cancer types:

1. Type 1 macrophages (M1).
2. Tumor-associated macrophages (TAMs).
3. B cells and plasma cells.
4. T cells.

**?** **Question 11**

For the preparation of dendritic cell (DC)-based vaccines:

1. Knockout mice are used.
2. The immunization of volunteers is exploited.
3. Transfection of DCs with RNA encoding a specific cancer antigen is used.
4. Vaccine BCG is employed.

**?** **Question 12**

In addition, for the preparation of dendritic cell (DC)-based vaccines:

1. Autologous tumor lysates, or Class I HLA molecules associated with tumor antigen-derived peptides, are used.
2. Transgenic mice are exploited.
3. Vaccine BCG is used.
4. The immunization of volunteers is employed.

**?** **Question 13**

Cervical cancer is caused by:

1. *Hepatitis B virus*.
2. *Human herpesvirus 8 (HHV-8)*.
3. *Human papillomavirus (HPV)*.
4. *Epstein-Barr virus (EBV)*.

**?** **Question 14**

Kaposi's sarcoma is linked to:

1. Type 1 *herpes simplex virus (HSV)*.
2. *Epstein-Barr virus (EBV)*.
3. *Hepatitis C virus*.
4. *Human herpesvirus 8 (HHV-8)*.

**?** **Question 15**

CA19-9 is the serum cancer-specific marker of:

1. Gastric, pancreatic, gallbladder, and bile duct cancers.
2. Lung cancer.
3. Non-Hodgkin lymphoma.
4. Thyroid cancer.

**?** Question 16

Prostate-specific antigen (PSA) is the serum cancer-specific marker of:

1. Breast cancer.
2. Ovarian cancer.
3. Prostate cancer.
4. Colorectal cancer.

## 6.7 Immunology of Graft Rejection and Survival

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### Definitions

*Transplantation* is the grafting of organs or tissues from one individual to another.

The grafts are rejected by the immune system unless the recipient is tolerant to the donor's antigens or immunosuppressive therapy is performed.

*Graft-versus-host disease (GVHD)* is the state when the graft contains allogeneic hematopoietic stem cells of the bone marrow that attack the tissues of host recipient.

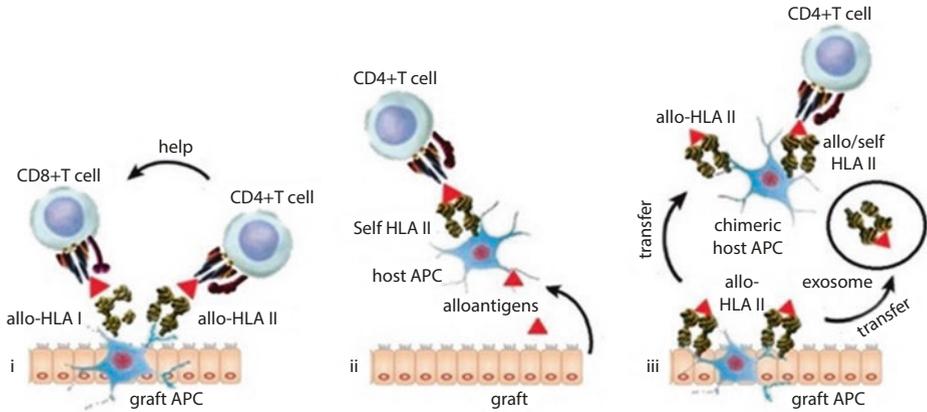
6

The immune system remains the most serious barrier to *transplantation* as a routine medical treatment. The intensity of immune responses to an allograft depends on the degree of HLA disparity between the donor graft and the recipient person. Autografts inside the same host (e.g., skin tissue) and isografts (between monozygotic twins) undergo no rejection. Xenografts from animals undergo rapid rejection.

Regardless of genetic disparity, the power and type of immune responses may vary from one individual to another depending on a variety of factors such as the site of transplantation, concomitant immunopathology, *cytomegalovirus (CMV)* infection, the donor's old age, and other conditions. Recognition of non-self-antigens by the immune system typically accompanies allograft transplantation and results in graft rejection.

**From a clinical viewpoint,** There are two modalities to prevent graft rejection, *immunosuppression* and *induction of allo-tolerance*. Induction and maintenance of immune tolerance is an important task in transplantation medicine and present research aims to design novel strategies to discover and develop approaches to induce specific tolerance.

There are two common cases in which any allograft may be accepted. If tissue or organ is grafted to an immunoprivileged site where antigens were always inaccessible for the immune system (e.g., in the eye or testes), the graft may survive. If in the case of any other graft transplantation, proper preparation for transplantation (i.e., from HLA-matched donors) is ensured and subsequent proper immunosuppressive therapy is performed, the graft will survive for a long time. Interestingly, a few patients can develop allograft tolerance upon cessation of all exogenous immunosuppressive treatment. The



■ Fig. 6.4 Three pathways of allorecognition during transplantation

unique gene signatures of these patients imply predisposition to immune tolerance. Also, liver transplantation creates a unique microenvironment that stimulates tolerance rather than immunity against the graft.

In transplantation, both types of T-cell-mediated responses and advanced B-cell-mediated response plus reactions of the innate immune system take place. However, there are three distinct pathways of allorecognition: (i) direct pathway, (ii) indirect pathway, and (iii) semidirect pathway (see ■ Fig. 6.4).

1. During the predominant direct pathway, host CD4+ and CD8+ T cells recognize intact allo-HLA molecules and allo-peptides on the surface of graft's antigen-presenting cells (APCs). Allo-HLA molecules are equivalent in shape to self-HLA molecules, but allo-peptides are foreign. Therefore T cells recognize the graft tissue as foreign antigens in a shortened manner, without the participation of self APCs. In this case, the number of differentiated effector T cells is extraordinarily high as compared to the number of T cells, which could proliferate after the presentation by self APCs. Effector T cells have enhanced reactivity against alloantigens due to cross-reactivity of the TCR with self- and allo-HLA molecules. The direct pathway of allorecognition is essential in *acute graft rejection*. The acute rejection manifests commonly in the first 6 months after transplantation.
2. During the indirect pathway, host CD4+ and CD8+ T cells recognize processed alloantigens presented as complexes self-HLA/allo-peptides by self APCs. After alloantigen recognition, T cells activate, proliferate, and differentiate into effector T cells. In this case, the antigenic repertoire is more variable, and there are no silent epitopes. The indirect pathway of allorecognition is important in *chronic graft rejection*. Chronic rejection develops after months or years.
3. During the semidirect pathway, host APCs with self-HLA molecules acquire intact allo-HLA molecules directly or/and through exosomes, and constitute chimeric APCs, which promote both direct and indirect pathways of allorecognition. Eventually, this type of pathway may be important in so-called subacute graft rejection.

During an advanced B-cell-mediated response, an accelerated number of alloantibodies are produced, which plays a role in the development of *hyperacute graft rejection and chronic graft vasculopathy*. In hyperacute rejection, the graft is rejected within minutes to hours due to the rapid destruction of vascularization.

Innate mechanisms alone do not appear sufficient to result in graft rejection itself. However, they are required for the effector phase of adaptive immunity against the graft and may play a significant role in resistance to tolerance induction.

There are some approaches in research to develop partial tolerance to graft alloantigens, which may be successful in the future. The fate of a graft, rejection or tolerance, depends on the relative balance between effector T cells and immunosuppressive nTreg. Th1 cells mediate allograft rejection, whereas Th2 cells protect allografts from tissue-destructive Th1 cells. In the past, Th2 cells were most prominent in grafts of tolerant patients. However, allograft rejection occurs in the absence of Th1 cells that may be caused by Th17 and other mediators. Modern tolerogenic therapeutic strategies differ in their capacity to directly delete effector T cells and preferentially promote the function and number of nTreg.

The use of immunosuppressive drugs and tissue-typing methods has increased the survival of allografts in the first year, but rejection cannot be now completely prevented yet. Furthermore, long-term use of immunosuppressive therapy leads to nephrotoxicity and metabolic disorders, as well as manifestations of immunocompromised conditions such as opportunistic infections and cancers. Immunosuppressive therapy is applied in two phases: (1) the initial induction phase, which requires much higher doses of immunosuppressive drugs, and (2) the later maintenance phase. Immunosuppressive medications in current use include the following:

- Inhibitors of calcineurin (tacrolimus and sirolimus) suppress the activation of T cells and cytokine production.
- Cyclophilin-binding agents (cyclosporine) inhibit T-cell signaling and actions of effector T cells.
- Synthetic isoxazole derivative drugs (leflunomide) have antiproliferative and antiviral activities.
- Antimetabolite drugs (azathioprine) downregulate DNA synthesis.
- Monoclonal antibodies are antagonists of IL2R (basiliximab and daclizumab), block IL2R, and prevent effects of IL2.

*Graft-versus-host disease (GVHD)* develops following allogeneic hematopoietic stem cell (bone marrow) transplantation under compromised conditions and may be acute (in the first 100 days) and chronic (after 100 days).

After bone marrow transplantation, T cells in the graft attack the tissues of the host recipient since they recognize the host tissues as antigenically foreign. The T cells produce an excess of pro-inflammatory cytokines, including TNF $\alpha$  and IFN $\gamma$ . GVHD can occur even when HLA-identical siblings are the donors. HLA-identical siblings or HLA-identical unrelated donors often have genetically different proteins (called minor histocompatibility antigens) that can be presented by HLA molecules to the donor's T cells. These antigens thus trigger adaptive immune responses.

The standard of care in acute and chronic GVHD is the intravenous administration of glucocorticoids.

**■ Quiz**

Reading a question, please choose only one right answer.

**? Question 1**

As a rule, the graft transplantation to an immunoprivileged site leads to:

1. Graft's rejection.
2. An allergic reaction.
3. Graft's survival.
4. An immunodeficiency.

**? Question 2**

The graft transplantation from HLA-matched donors results in:

1. Graft's survival for a long time.
2. Acute graft's rejection.
3. An immunodeficiency.
4. Subacute graft's rejection.

**? Question 3**

The acute graft's rejection manifests:

1. In first 2 months after transplantation.
2. After months and years.
3. In first 6 months after transplantation.
4. Immediately.

**? Question 4**

The chronic graft's rejection manifests:

1. In first 6 months after transplantation.
2. After months and years.
3. Never.
4. In first 3 months after transplantation.

**? Question 5**

During the semidirect rejection pathway, host antigen-presenting cells with self-HLA molecules:

1. Present complexes self-HLA/allo-peptides to lymphocytes.
2. Constitute chimeric antigen-presenting cells with allo-HLA molecules.
3. Undergo apoptosis.
4. Are not involved.

**? Question 6**

During the direct rejection pathway, host antigen-presenting cells with self-HLA molecules:

1. Constitute chimeric antigen-presenting cells with allo-HLA molecules.
2. Undergo apoptosis.
3. Present complexes self-HLA/allo-peptides to lymphocytes.
4. Are not involved.

**? Question 7**

Hyperacute graft rejection and chronic graft vasculopathy are caused by:

1. CD4+ T cells.
2. CD8+ T cells.
3. Alloantibodies.
4. Phagocytes.

**? Question 8**

The liver transplantation stimulates:

1. Immune tolerance rather than immune rejection.
2. Subacute rejection.
3. Chronic rejection.
4. Chronic graft vasculopathy.

**6****? Question 9**

Innate mechanisms alone are sufficient to result in graft rejection:

1. Yes.
2. No.
3. Probably.
4. Unknown.

**? Question 10**

Type 1 helper T cells:

1. Downregulate the graft's rejection.
2. Potentiate the graft's rejection.
3. Their role is unknown.
4. Stimulate tolerance.

**? Question 11**

Type 2 helper T cells:

1. Potentiate the graft's rejection.
2. Their role is unknown.
3. Themselves damage to graft's tissue.
4. Downregulate the graft's rejection.

**? Question 12**

Type 17 helper T cells:

1. Downregulate the graft's rejection.
2. Potentiate the graft's rejection.
3. Stimulate tolerance.
4. Their role is unknown.

**? Question 13**

Nonadaptive nTreg:

1. Downregulate the graft's rejection.
2. Their role is unknown.
3. Themselves damage to graft's tissue.
4. Potentiate the graft's rejection.

**? Question 14**

In the graft transplantation the main therapeutic approach is:

1. Antibiotic therapy.
2. Immunosuppressive therapy.
3. Immune enhancement therapy.
4. Allergen-specific immunotherapy.

**? Question 15**

Graft-versus-host disease (GVHD) may occur in:

1. Bone marrow transplantation under compromised conditions.
2. Thymus transplantation.
3. Transplantation to an immunoprivileged site.
4. Liver transplantation under compromised conditions.

**? Question 16**

The standard of treatment in GVHD is:

1. Immune enhancement therapy.
2. Glucocorticoids per os.
3. Antibiotic therapy.
4. Glucocorticoids intravenously.

**Key Points**

1. Immunopathology is the group of the immune system dependent diseases, syndromes, and short-term reactions in humans. In some cases, the immune system may function either in an insufficient manner (immunodeficiency), or in a deviated fashion (allergy), or in an excessive regimen (autoimmunity), or on a "path of betrayal" (cancer).
2. Immunodeficiencies are caused by gene mutations, harmful environmental factors, viruses, chemotherapy, stress, etc. and manifest themselves as peracute or chronic, often unusual infections. Breakdown in the natural tolerance to self-antigens results in an array of autoimmune diseases. Recently described autoinflammatory disorders originate from the imbalance of innate immunity. *HIV* and cancer may be taken into consideration as the combined forms of immunopathology.
3. Nowadays, allergy is a highly spread set of immunopathology, which is characterized by the pathogenic and clinical polymorphism, chronic course, difficulties in the treatment, and the poor prognosis in some cases. Atopic diseases are the predominant allergic conditions.

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# Immunology of Infectious Processes

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## Learning Objectives

*Knowledge.* Upon successful completion of the chapter, students should be able to:

1. List and characterize three classes of microbes, which are related to evolutionary constituted interrelationships between human beings and microbes.
2. Explain the reactivation of opportunistic germs and its clinical significance.
3. Compare and contrast the predominance of an immunological mechanism depending on the peculiarity of a microbe.

*Acquired Skills.* Upon successful completion of the chapter, students should demonstrate following skills, including:

1. Interpret the knowledge related to the immunology of infectious processes.
2. Critically evaluate the scientific literature about the immunology of infectious processes.
3. Discuss the scientific articles from the current research literature to criticize experimental and clinical data and formulation of new hypotheses in clinical immunology and infectious diseases.
4. Attain a clear perception of the presented immunology definitions expressed orally and in written form.
5. Formulate the introduced immunology terms.
6. Correctly answer quiz questions.

*Attitude and Professional Behaviors.* Students should be able to:

1. Have the readiness to be hardworking.
2. Behave professionally at all times.
3. Recognize the importance of studying and demonstrate a commitment.

## 7.1 Introduction

The human body exists along with the microbiota, which may weigh some kilograms. There are numerous exciting details related to the interaction between symbiotic, opportunistic, or absolutely pathogenic microbes and the human immune system at both physiological and pathologic levels.

### Definitions

*Infection* is the invasion of external *absolutely pathogenic microbes* such as viruses, bacteria, fungi, protozoans, etc. or reactivation of *opportunistic germs* living in barrier tissues of the body.

Evolutionarily constituted interrelationships between human beings and microbes may be categorized into three classes as follows:

- *Mutualism.* This term describes an association in which both microbes and the human body undoubtedly benefit. Symbiotic microbes of the human microbiome may belong to this class.



immunity and advanced immune responses. The constituted individual microbiota is supported by the immune system as a part of “self” during one’s whole life and is linked to self-tolerance. On the other hand, the opportunistic microbes are under permanent immunological surveillance.

However, the opportunistic microbes of the normal microbiota may be reactivated under a number of conditions. This event is called *reactivation*. The states that may result in the reactivation of opportunistic microbes are as follows:

1. Weakening of the immune system
2. Imbalance in the microbiota when there is a decrease in a number of symbiotic microbes and other microbes, which are antagonistic to reactivated ones
3. Translocation of the opportunistic microbes to uncommon sites
4. Superinfection with the opportunistic microbes

There are three types of states of the opportunistic microbes in the body (see ■ Table 7.1).

Absolutely pathogenic germs and some opportunistic microbes cause infectious diseases when they invade the human body or are reactivated. In these cases, all immunological mechanisms are involved in protection against them, including the innate immune system and adaptive immune responses, and the predominance of an immunological mechanism depends on the evolutionally constituted localization of the parasitic microbes in the body (see ■ Table 7.2). A laboratory criterion of the reactivation of the opportunistic microbes is the appearance of specific IgM antibodies in the blood.

The innate immune system, including “acute phase” proteins, complement, phagocytosis, interferons, NK cells, etc., is mobilized very rapidly after exposure to absolutely pathogenic microbes or reactivation of opportunistic microbes, and the antimicrobial effects may be very efficient, but they are short term and do not form memory. The innate immune system exhibits:

- *Immune clearance or immune containment* when absolutely pathogenic microbes affect the body
- *Immune containment* when opportunistic microbes are reactivated

■ Table 7.1 Types of reactivation of the opportunistic microbes

Feature	Latent state	Subclinical reactivation	Clinical reactivation
Sites of the opportunistic microbes	Common sites (tissues, cells), where the immune containment takes place	Secretions	Blood, CNS, peripheral nerves, skin, mucosae, liver, eyes, etc.
Clinical importance	Neither clinical nor epidemiological importance	No clinical importance (treatment is not required), but there is epidemiological value, and the spread of the microbes is possible	There is clinical importance (treatment is required) and epidemiological value

■ **Table 7.2** Predominance of an immunological mechanism

Part of a pathogen and its location	Immunological mechanism
All pathogens ( <i>patterns</i> )	Innate immunity
Extracellular pathogens ( <i>patterns</i> )	Simple B-cell-mediated adaptive response
Extracellular pathogens ( <i>antigens</i> )	Advanced B-cell-mediated adaptive response with constituted memory
Intracellular pathogens – viruses ( <i>antigens</i> )	CD8+ T-cell-mediated adaptive response with constituted memory
Other intracellular pathogens ( <i>antigens</i> )	CD4+ T-cell-mediated adaptive response with constituted memory

Adaptive immunity, including antibodies, CD8+ T cells, and CD4+ T cells, may function at three levels:

- *Defensive level* when there is the *immune clearance* of the absolutely pathogenic microbes and *immune containment* of the reactivated opportunistic microbes
- *Witness level* when both the *eradication* and *deterrence* of infection are *not achieved*
- *Pathologic level* in the cases of cross-reactions of microbial antigens and self-antigens and, in total, in *autoimmune disorders*

The absolutely pathogenic infections may be acute and chronic. Reactivation of the opportunistic microbes may sometimes be wave-relapsing (see ■ Fig. 7.2 and ■ Table 7.3).

Under immunocompromised conditions, the absolutely pathogenic infections may be very severe up to peracute forms or may become chronic, whereas reactivation of the opportunistic microbes in the body may also be either severe or permanent or wave-relapsing.

**From a clinical viewpoint,** there are two main therapeutic approaches to defend the body from infections: (1) antimicrobial therapy and (2) immune enhancement therapy. In most cases of opportunistic microbiota's reactivation, clinical symptoms and correspondingly problem for any physician are present, whereas the opportunistic antigens remain low immunogenic, even tolerogenic as if these microbes were in the resting state. Hence, the containment due to adaptive immunity is not developing. In general practice, physicians commonly prescribe antibiotic and antiviral medications but not immune enhancement therapy including adjuvant therapy, which must be the principal strategy to solve both the reactivation episode and whole problem. Therefore, such medical disorders as recurrent respiratory diseases, skin disease, sexual *herpes simplex virus* infections, bacterial vaginosis, candidiasis, and so on remain to spread worldwide.

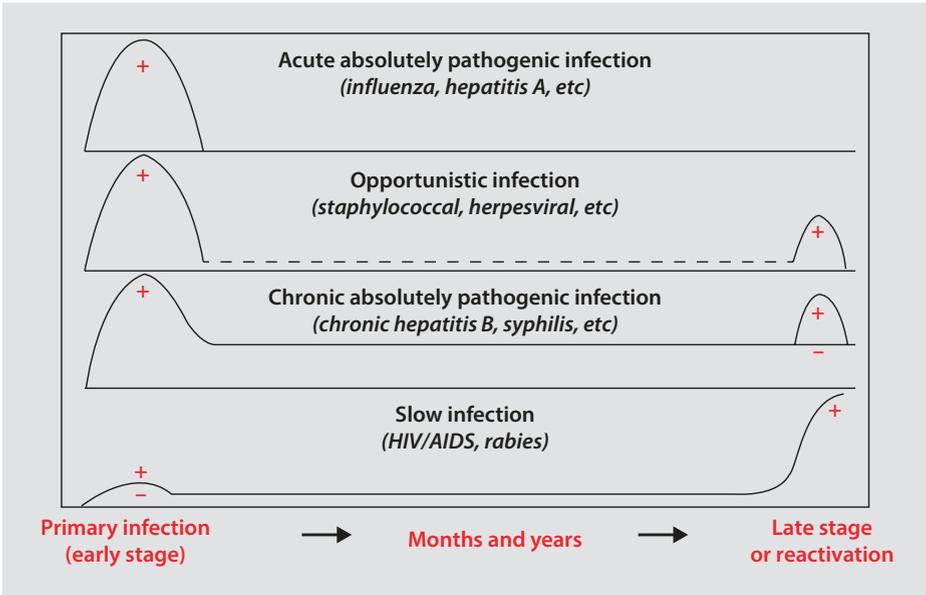


Fig. 7.2 Different types of infection

Table 7.3 Immunological phenomenons in different types of the infections

Infection type	Immunological outcomes
Parts of the normal non-reactivated microbiome	Absence of reactions from the innate immune system Natural tolerance
Acute absolutely pathogenic infection	Reactions from the innate immune system Immune clearance due to the adaptive responses
Chronic absolutely pathogenic infection (including slow infection with no recovery)	Defects of the innate immune system Pathologic tolerance
Acute opportunistic infection (primary entry)	Reactions from the innate immune system Immune containment
Wave-relapsing reactivation of opportunistic infection (often reactivation episodes)	Defects of the innate immune system Noneffective immune responses and pathologic tolerance

New absolutely pathogenic microbes, unknown to the human immune system and potentially pandemic, may be originated due to (1) spontaneous mutations in the microbial genomes (e.g., influenza viruses), (2) mutations leading to the drug resistance to known antimicrobial medications (e.g., *Mycobacterium tuberculosis*), (3) transmis-

sion from animals (e.g., avian influenza viruses like H5N1, *Zaire ebolavirus*, and prions), (4) return of microbes of the past (e.g., *Pasteurella pestis* and *smallpox virus*), (5) technogenic factors (e.g., *Legionella pneumophila* in air conditioning systems), and (6) unspecified causes (e.g., *HIV*).

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

Symbiotic microbes are in the interrelationship with human beings, which may be categorized like:

1. Antagonism.
2. Commensalism.
3. Mutualism.
4. Parasitism.

#### ? Question 2

Non-reactivated opportunistic microbes are in the interrelationship with human beings, which may be categorized like:

1. Commensalism.
2. Mutualism.
3. Parasitism.
4. Antagonism.

#### ? Question 3

Reactivated opportunistic microbes are in the interrelationship with human beings, which may be categorized like:

1. Antagonism.
2. Commensalism.
3. Mutualism.
4. Parasitism.

#### ? Question 4

The human microbiome is made up of:

1.  $10^{14}$  microbes.
2.  $10^5$  microbes.
3.  $10^{10}$  microbes.
4.  $10^{25}$  microbes.

#### ? Question 5

The baby's skin and mucosal microbiota trigger the education and maturation of:

1. The central nervous system.
2. The immune system.
3. The endocrine glands.
4. The liver.

**? Question 6**

Human microbiota stabilizes in composition and number after:

1. 40 years.
2. The age of puberty.
3. About 3 years.
4. 10 years.

**? Question 7**

This factor does not lead to the reactivation of opportunistic microbes:

1. Weakening of the immune system.
2. Superinfection with them.
3. Immune enhancement therapy.
4. Translocation of them to uncommon sites.

**7****? Question 8**

This factor also does not result in the reactivation of opportunistic microbes:

1. Translocation of them to uncommon sites.
2. Imbalance in the microbiota due to a decrease in a number of symbiotic microbes.
3. Immunization.
4. Weakening of the immune system.

**? Question 9**

The body protects from viruses by means of:

1. CD4+ T-cell-mediated adaptive response.
2. CD8+ T-cell-mediated adaptive response.
3. Phagocytosis.
4. Lysozyme.

**? Question 10**

The body protects from intracellularly located bacteria by means of:

1. Histamine.
2. CD4+ T-cell-mediated adaptive response.
3. Simple B-cell-mediated adaptive response.
4. CD8+ T-cell-mediated adaptive response.

**? Question 11**

The body defends against extracellular pathogens by:

1. CD8+ T-cell-mediated adaptive response.
2. CD4+ T-cell-mediated adaptive response.
3. B-cell-mediated adaptive responses.
4. NK cells and interferons.

**? Question 12**

All the “patterns” trigger:

1. B cells.

2. T cells.
3. Thymus involution.
4. Reactions of the innate immunity.

**?** **Question 13**

Deterrent effectors lead to:

1. Immunopathology.
2. A halt of reactivated opportunistic microbes.
3. "Acute phase" reaction.
4. The immune clearance.

**?** **Question 14**

Pathogenic effectors are:

1. "Witness antibodies."
2. Autoantibodies and autoreactive T cells.
3. Effectors, which are able to fulfill the immune clearance.
4. Effectors, which are able to fulfill the immune containment.

**?** **Question 15**

Chronic absolutely pathogenic infection (including slow infection with no recovery) is characterized by:

1. Defects of the innate immune system and pathologic tolerance.
2. Natural tolerance.
3. Immune containment.
4. Immune clearance due to the adaptive responses.

**?** **Question 16**

Acute opportunistic infection is characterized by:

1. Reactions from the innate immune system and immune containment.
2. Immune clearance due to the adaptive responses.
3. Natural tolerance.
4. Autoimmune disorders.

**Key Points**

1. Fate of both absolutely pathogenic microbes and reactivated opportunistic germs depends on the efficacy of functioning of immune system in a single case and evolutionary constituted interrelationships between human beings and microbes.
2. If immunodeficiency is absent, there are two end effects of the innate and adaptive immunity, immune clearance and immune containment. In total, the infectious process may result in recovery, progressive wave-relapsing, chronic course of the infection, or even patient's death.

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# Vaccination

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## Learning Objectives

*Knowledge.* Upon successful completion of the chapter, students should be able to:

1. Explain the rationale for vaccination. Define the herd immunity.
2. Be familiar with the recommended immunization schedule.
3. Distinguish between the contraindications and relative contraindications to vaccination.
4. Describe types of the classically manufactured and genetically engineered vaccines.
5. Name and characterize the side effects associated with immunization.

*Acquired Skills.* Upon successful completion of the chapter, students should demonstrate the following skills, including:

1. Interpret the knowledge related to vaccines and vaccination.
2. Critically evaluate the clinical literature about vaccination.
3. Discuss the scientific articles from the current research literature to criticize experimental and clinical data and formulation of new hypotheses in clinical immunology/allergy and infectious diseases.
4. Attain a clear perception of the presented immunology definitions expressed orally and in written form.
5. Formulate the submitted immunology terms.
6. Correctly answer quiz questions.

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*Attitude and Professional Behaviors.* Students should be able to:

1. Have the readiness to be hardworking.
2. Behave professionally at all times.
3. Recognize the importance of studying and demonstrate a commitment.
4. Demonstrate the consideration of the patient's feelings, ethnic, religious, cultural, and social background, and be able to display empathy.

## 8.1 Introduction

Vaccination leads to the artificial immunity not entirely analogous to natural immune memory and herd immunity. The reader can find medical principles of immunization including recommended schedule, indications, contraindications, side effects and emergency procedures, types of vaccines, and strategies for the development of new vaccines.

### Definitions

*Vaccination or immunization* is a preventive medical approach, which causes the formation of artificial immunity against antigens included in used vaccine.

*Herd immunity* is a form of indirect protection against infection in non-vaccinated individuals that occurs when most of the population has immune memory to the infection due to the vaccination.

The history of inoculation is long. Already in the eighteenth century did British doctor Edward Jenner manage to establish the vaccination procedure by introducing material

from a cowpox vesicle, obtain a positive result for immunity against smallpox, and later create widespread interest in the vaccination.

Normally, up-to-date vaccines induce adaptive immune responses and provide the body with immune memory for a long-term period in human life to those pathogens against which they have been directed. One type of vaccine manufactured of antigens from extracellularly located pathogens can induce an advanced B-cell-mediated response, formation of specific *antibodies* and long-term memory B cells, and the preventive effect of *immune clearance*. Another type of vaccines is manufactured of antigens from intracellularly parasitized pathogens. It can trigger CD4+ and CD8+ T-cell-mediated responses, the formation of *effector CD4+ T cells*, *CD8+ T cells*, and long-lasting memory CD4+ and CD8+ T cells and achieve preventive effects of *immune clearance*.

However, there is a third type of vaccines made of antigens from opportunistic pathogens that may result in the establishment of *immune containment* only and short-term immune memory. Unfortunately, artificial immunity after all types of vaccines is not entirely identical as compared to natural immunity, which is always lifelong against absolutely pathogenic microbes.

Furthermore, there are questions related to the immunological safety of vaccines and their capacity to trigger non-antigen-specific responses possibly leading to conditions such as allergies, autoimmunity including pathology of the CNS, or even premature death. Additionally, some parents choose not to vaccinate their children because of fear of autism or other disorders. Nowadays, groups of activists, antivaccinationists, object to the scientific basis, medical safety, religious ground, ethical aspect, and legislative right of any vaccination. It leads to a decrease in *herd immunity* (community immunity, population immunity, or social immunity), which is a form of indirect protection from infectious disease that occurs when a large percentage of a population has become immune to infectious agents, thereby providing a measure of protection for individuals who are not immune.

Nowadays, three million deaths per year are prevented worldwide by vaccinations. To date, the eradication of smallpox is one of the most significant achievements of modern medicine. It became possible due to an effective vaccine and global vaccination programs, which led to herd immunity to the infection.

There are two goals of any vaccination, tactical and strategic. The tactical goal is the generation of long-lived memory T cells and B cells to a particular infectious pathogen at the individual level, whereas the strategic goal is the achievement of herd immunity to this pathogen at the population level. Herd immunity is a form of indirect defense against infection in non-vaccinated individuals that occurs when most of the population has become immune to the infection due to the vaccination.

For each vaccine and each age, there is a recommended immunization schedule (see ■ Table 8.1).

There are *contraindications* against vaccination, which include:

- Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component
- Primary immunodeficiency (e.g., severe combined immunodeficiency, SCID)
- Known severe secondary immunodeficiency (e.g., from hematologic and solid tumors, receipt of chemotherapy, congenital immunodeficiency, or long-term immunosuppressive therapy or patients with HIV who are severely immunocompromised)

**Table 8.1** Recommended immunization schedule for children and adolescents aged 18 years or younger, United States, 2018 (see site at ► [www.cdc.gov/vaccines/](http://www.cdc.gov/vaccines/))

Birth to 15 months

Vaccines	Birth	Months						
		1	2	4	6	9	12	15
Hepatitis B (HepB)	1 <sup>st</sup> dose	2 <sup>nd</sup> dose			3 <sup>rd</sup> dose			
Rotavirus (RV1 (2-dose series) or RV5 (3-dose series))			1 <sup>st</sup> dose	2 <sup>nd</sup> dose	3 <sup>rd</sup> dose			
Vaccines	Birth	Months						
		1	2	4	6	9	12	15
Diphtheria, tetanus, acellular pertussis (DTaP<7 yrs)			1 <sup>st</sup> dose	2 <sup>nd</sup> dose	3 <sup>rd</sup> dose			4 <sup>th</sup> dose
<i>Haemophilus influenzae type b (Hib)</i>			1 <sup>st</sup> dose	2 <sup>nd</sup> dose	3 <sup>rd</sup> dose (if present in primary series)		3 <sup>rd</sup> or 4 <sup>th</sup> dose (secondary series)	
Pneumo-coccal conjugate (PCV13)			1 <sup>st</sup> dose	2 <sup>nd</sup> dose	3 <sup>rd</sup> dose		4 <sup>th</sup> dose	
Inactivated poliovirus (IPV<18 yrs)			1 <sup>st</sup> dose	2 <sup>nd</sup> dose	3 <sup>rd</sup> dose			
Influenza (IIV)					Annual vaccination (IIV) 1 or 2 doses			
Measles, mumps, rubella (MMR)							1 <sup>st</sup> dose	
Varicella (VAR)							1 <sup>st</sup> dose	
Hepatitis A (HepA)							2-dose series	

■ **Table 8.1** continued

Meningo- coccal MenCY- Hib≥6 weeks MenACWY- D≥9 months MenACWY- CRM≥2 months			For children with high-risk conditions
--	--	--	--

15 months to 18 years

Vaccines	Months		Years						
	18	19-23	2-3	4-6	7-10	11-12	13-15	16	17-18
Hepatitis B (HepB)	3 <sup>rd</sup> dose								
Diphtheria, tetanus, acellular pertussis (DTaP<7 yrs)	4 <sup>th</sup> dose			5 <sup>th</sup> dose					
Inactivated poliovirus (IPV<18 yrs)	3 <sup>rd</sup> dose			4 <sup>th</sup> dose					
Influenza (IIV)	Annual vaccination (IIV) 1 or 2 doses				Annual vaccination (IIV) 1 dose only				
Measles, mumps, rubella (MMR)				2 <sup>nd</sup> dose					
Varicella (VAR)				2 <sup>nd</sup> dose					
Hepatitis A (HepA)	2-dose series								
	(if not vaccinated previously)								

(continued)

Table 8.1 continued

Meningo- coccal MenCY-Hib≥6 weeks MenACWY- D≥9 months MenACWY- CRM≥2 months	For children with high-risk conditions					1 <sup>st</sup> dose			2 <sup>nd</sup> dose	
Tetanus, diphtheria, acellular pertussis (Tdap<7 yrs)						Tdap				
Vaccines	Months		Years							
	18	19-23	2-3	4-6	7-10	11-12	13-15	16	17-18	
<i>Human Papilloma- virus (HPV)</i>						2- dose series		3-dose series (if was not vaccinated previously)		
Meningo- coccal B					For children with high-risk conditions					
Pneumo- coccal polysaccha- ride (PPSV23)			According to special indications							

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- For *Bordetella pertussis*-containing vaccines, encephalopathy (e.g., coma, decreased level of consciousness, prolonged seizures)
- For *Haemophilus influenzae type b* (Hib), age younger than 6 weeks

Relative contraindications against vaccination include:

- Infant weighing less than 2000 grams
- Moderate or severe acute illness with or without fever
- For diphtheria and tetanus toxoid, temperature of 105°F or higher (40.5 °C or higher); collapse or shock-like state; persistent, inconsolable crying lasting 3 or more hours within 48 h; seizure within 3 days after receiving a previous dose; and history of type III hypersensitivity reactions after a previous dose of tetanus or diphtheria toxoid-containing vaccine
- For diphtheria and tetanus toxoid, pertussis, and influenza, history of Guillain-Barré syndrome within 6 weeks of previous vaccination
- For measles, mumps, rubella (MMR), recent (within 11 months) receipt of antibody-containing blood product, history of thrombocytopenia or thrombocytopenic purpura, and need for tuberculin skin testing

Gradually, the history of vaccine development led to the creation of several types of vaccines:

- Live (attenuated) vaccines
- Inactivated (killed) vaccines
- Toxoid vaccines
- Subunit vaccines (chemical, recombinant, conjugate, and virus-like particle (VLP) subunit vaccines)
- Recombinant vector vaccines
- DNA vaccines

However, classically manufactured vaccines such as live, inactivated, and toxoid vaccines have several disadvantages. For *live (attenuated) vaccines* (e.g., against measles, rubella, and mumps), the genetic lesion responsible for the attenuation of the pathogen remains mostly undefined, harboring a risk of reversion and vaccine-induced diseases. For *inactivated (killed)* (e.g., against polio, hepatitis A, influenza, and rabies) and *toxoid vaccines* (e.g., against tetanus and diphtheria), there is a serious risk of reversion of the toxoid to its toxigenic form, and it always remains possible that the inactivation of pathogens within the preparation process is not complete.

Genetically engineered vaccines such as subunit, recombinant vector, and DNA vaccines are able to solve some of these problems. However, new issues are arising.

The *subunit vaccines* contain only the essential antigens or antigenic epitopes, not the whole microbes; therefore they are safer than other types of vaccines. Examples of the subunit vaccines include the vaccines against *Hepatitis B virus*, *influenza virus*, *H. influenzae type b*, *human papillomavirus (HPV)*, etc. Researchers are developing a recombinant subunit vaccine against hepatitis C virus. The subunit vaccines may comprise up to 20 or more antigens and are manufactured in four ways:

1. Chemically splitting the whole microbes and using the separate proteins (chemical subunit vaccines, e.g., acellular vaccine against pertussis and hepatitis B).
2. Synthesis of antigens by recombinant DNA technology (recombinant subunit vaccines, e.g., some vaccines against seasonal influenza).
3. Conjugation of poorly immunogenic polysaccharides, which coat bacteria (e.g., *H. influenzae type b*, *meningococci*, and *pneumococci*), with toxoid to enhance immunogenicity (conjugate subunit vaccines).
4. The principle of virus-like particle (VLP) when use is made of the intrinsic ability of some viral capsid and envelope proteins to self-organize in VLP without other viral components including the viral genome (VLP subunit vaccines, e.g., *HPV* vaccine).

However, there are some disadvantages of the subunit vaccines including lower immunogenicity, shorter duration of the immunity, and requirement in strong adjuvants and linkage to carriers.

The *recombinant vector vaccines* are still in the experimental stage. They apply an attenuated virus or bacterium to introduce microbial DNA to cells of the body. “Vector” relates to the harmless attenuated virus or bacterium into which the antigen-encoding genes of a pathogen are inserted to exploit the vector as the carrier of the target microbe

that allows the target microbial DNA to fall into cells of the human body. Recombinant vector vaccines strongly mimic a natural infection and therefore may trigger the effective adaptive immune responses. To date, researchers are working on viral-based recombinant vector vaccines against *HIV*, rabies, and measles. However, there are two disadvantages of recombinant vector vaccines: (1) they do not mimic the virion surface because the recombinant antigen is expressed on the surface of the body cells, and (2) the attenuated vector may become harmful in immunocompromised persons.

The *DNA vaccines* are still in the experimental stage, but certain ones against influenza and herpes simplex are being trialed in humans. If the genes for a microbe's antigens are introduced into the body, the body's self-cells become vaccine-making factories, synthesizing the antigens required to trigger the adaptive immune responses. DNA vaccines are safe because they do not comprise the microbes but just a few copies of them. There are also some disadvantages of DNA vaccines including the possibility of tolerance to the produced antigens, the formation of autoantibodies, action on genes controlling cell growth, and restriction of used antigens (proteins only).

Nowadays, researchers are continuing work on new types of vaccine, e.g., dendritic cell vaccines. Some such vaccines are already in use. *Dendritic cell-based vaccines* are mainly used in cancer immunotherapy. In order to trigger a CD8+ T-cell-mediated immune response and other types of responses in cancer patients, dendritic cells (DCs) have to present the relevant tumor antigens. For this purpose, either the preparation of autologous tumor lysates, or Class I HLA molecules associated with tumor antigen-derived peptides, or transfection of DCs with RNA encoding a specific cancer antigen is used. At the next stage, DCs with antigens of interest should become mature. Subsequently, DCs may be administered to a cancer patient via intradermal, intravenous, or intralymphatic injection. However, concerning DC-based vaccines, there is still a variety of unresolved problems.

In practice, it is useful to understand to which vaccine type any vaccine belongs (see

■ Table 8.2).

**From a clinical point of view,** analogous to medications, there are possible risks and *side effects* associated with vaccines. However, the occurrence of severe allergic reaction is sporadic. In comparison, the risk of severe complications, hospitalization, or death from vaccine-preventable disease is much higher. The benefits of vaccination far outweigh the risks.

According to the Centers for Disease Control and Prevention (CDC), in most cases, vaccine side effects are minor and go away within a few days. Side effects vary according to the vaccine type and may include:

- Pain, redness, tenderness, itching or swelling at the injection site
- Weakness
- Headache
- Nausea
- Fever
- Mild rash

Serious side effects of vaccination are rare but may occur. Severe reactions can include anaphylaxis, Guillain-Barré syndrome, high fever, heart problems, difficulty breathing, hoarseness or wheezing, hives, and angioedema. See ■ Table 8.3 on how to perform emergency procedures.

■ **Table 8.2** Some vaccines used in the United States, European Union, and Russia

Vaccine's name	Infections	Type of vaccine
HepB (Engerix-B, Recombivax HB)	Hepatitis B	Protein
DTaP-HepB-IPV (Pediatrix)	Diphtheria, tetanus, pertussis, hepatitis B, polio	Toxoid, toxoid, protein, protein, inactivated
HepA-HepB (Twinrix)	Hepatitis A, hepatitis B	Inactivated, protein
HepA (Havrix, Vaqta)	Hepatitis A	Inactivated
DTaP-IPV (Kinrix, Quadracel)	Diphtheria, tetanus, pertussis, polio	Toxoid, toxoid, protein, inactivated
DTaP-IPV/Hib (Pentacel)	Diphtheria, tetanus, pertussis, polio, <i>Haemophilus influenzae type b</i>	Toxoid, toxoid, protein, inactivated, conjugated
DTaP (Daptacel, Infanrix), Tdap (Adacel, Boostrix)	Diphtheria, tetanus, pertussis	Toxoid, toxoid, protein
Polio (Ipol)	Polio	Inactivated
Hib (ActHIB, PedvaxHIB, Hiberix)	<i>Haemophilus influenzae type b</i>	Conjugated
MenCY-Hib (MenHibrix)	Meningococcal, <i>Haemophilus influenzae type b</i>	Conjugated, conjugated
MMR (M-M-R II)	Measles, mumps, rubella	Attenuated, attenuated, attenuated
MMRV (ProQuad)	Measles, mumps, rubella, varicella	Attenuated, attenuated, attenuated, attenuated
RV1 (Rotarix) RV5 (RotaTeq)	<i>Rotavirus</i>	Attenuated
HPV9 (Gardasil 9) HPV4 (Gardasil) HPV2 (Cervarix)	<i>Human Papilloma Virus (HPV)</i>	VLP
PCV13 (Pneumovax 13) PPSV23 (Pneumovax 23)	Pneumococcal	Conjugated
MenACWY (Menactra, Menveo) MPSV4 (Menomune) MenB (Bexsero, Trumenba)	Meningococcal	Conjugated
VAR (Varivax)	Varicella	Attenuated
IIV (Afluria, Flud, Flublok, Flucelvax, FluLaval, Fluarix, Fluvirin, Fluzone, Fluzone High-Dose, Fluzone Intradermal)	Seasonal influenza (flu)	Inactivated and recombinant subunit

**Table 8.3** Emergency procedures in severe side effects upon vaccination

Clinical signs	Treatment
Skin rash (flush, erythema, urticaria, angioedema, etc.)	(1) Antihistamines (diphenhydramine (Benadryl®)) – adults, 25 mg per os in 6 h for 2–5 days; children, 1 mg/kg per os in 8 h for 2–5 days (2) Corticosteroids (prednisone) – adults, 20–80 mg per os daily for 2–5 days; children, 0.5–1 mg/kg per os daily for 2–5 days
Fall in blood pressure (<90 systolic)	Epinephrine 0.25–0.5 mL of the ten times diluted standard solution 1–2 times/h 0.25–0.5 mL subcutaneously
Shock, dyspnea	(1) Epinephrine 0.25–0.5 mL of the ten times diluted standard solution slowly intravenously, possibly repeated at intervals of 2–3 min (2) Corticosteroids, e.g., prednisone 250–1000 mg intravenously (3) Volume replacement, preferentially with a 5% human albumin solution
Bronchospasm	Inhalation of $\beta_2$ -adrenergic agonists such as albuterol
High fever	Fevers only need to be treated with medicine if they cause discomfort. Most often, this means fevers above 39 °C (102 °F) For fevers above 39 °C (102 °F), acetaminophen product (such as Tylenol®) must be given Another choice is an ibuprofen product (such as Advil®)

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

All up-to-date vaccines induce the formation of:

1. Immune memory for a short-term period.
2. Lifelong immune memory.
3. Immune memory for a long-term period.
4. IgE.

#### ? Question 2

Vaccines made of antigens from opportunistic pathogens can provide:

1. The immune containment if opportunistic microbes are reactivated.
2. The immune clearance.
3. The permanent activation of innate immunity.
4. Lifelong immune memory.

#### ? Question 3

Vaccines manufactured from antigens from extracellular pathogens mainly induce:

1. CD8+ T-cell-mediated adaptive response.
2. CD4+ T-cell-mediated adaptive response.
3. Reactions of the innate immunity.
4. Advanced B-cell-mediated adaptive response.

**? Question 4**

Vaccines manufactured of antigens from intracellular pathogens mainly induce:

1. Reactions of innate immunity.
2. T-cell-mediated adaptive responses.
3. Simple B-cell-mediated immune response.
4. Advanced B-cell-mediated immune response.

**? Question 5**

Herd immunity is:

1. Vaccination in the enlarged sense.
2. The population immunity on the base of most vaccinated persons in the community.
3. Protective immunity due to vaccination.
4. All types of immunity.

**? Question 6**

Vaccine HepB induces the protective immunity against:

1. Hepatitis C.
2. Pertussis and polio.
3. Hepatitis B.
4. *Haemophilus influenzae type b*.

**? Question 7**

Vaccine DTaP creates the protective immunity against:

1. Measles, mumps, and rubella.
2. Pertussis, hepatitis B, and polio.
3. Diphtheria, tetanus, and pertussis.
4. Hepatitis A, B, and C.

**? Question 8**

Vaccine MMRV contains:

1. Attenuated viruses of measles, mumps, rubella, and varicella.
2. Attenuated *Poliovirus*.
3. Staphylococcal toxoid.
4. Conjugated meningococcal polysaccharides with toxoid.

**? Question 9**

This type of vaccines is not related to the genetically engineered vaccines:

1. Subunit vaccines.
2. Attenuated vaccines.
3. DNA vaccines.
4. Recombinant vector vaccines.

**? Question 10**

This type of vaccines is not related to the subunit vaccines:

1. Chemical vaccines.
2. VLP vaccines.
3. DNA vaccines.
4. Conjugated vaccines.

**? Question 11**

Regarding to vaccines and vaccination, VLP means:

1. Very late protein.
2. Very low percentage.
3. Virus-like particle.
4. Vaccine like protein.

**? Question 12**

A contraindication to vaccination with live vaccines is:

1. Primary immunodeficiency.
2. Atopic dermatitis.
3. Bronchial asthma.
4. Seasonal allergic rhinitis.

**? Question 13**

A severe side effect of vaccination is:

1. Headache.
2. Subfebrile fever.
3. Anaphylaxis.
4. Redness at injection site.

**? Question 14**

Effective *HIV* vaccine is available and widespread:

1. Yes.
2. In development only.
3. Unknown.
4. Will not be ever manufactured.

**? Question 15**

For the preparation of dendritic cell (DC)-based vaccines:

1. Transgenic mice are used.
2. The immunization of volunteers is exploited.
3. Transfection of DCs with RNA encoding a specific cancer antigen is used.
4. Vaccine BCG is employed.

**? Question 16**

In addition, for the preparation of dendritic cell (DC)-based vaccines:

1. Autologous tumor lysates or Class I HLA molecules associated with tumor antigen-derived peptides are used.
2. Knockout mice are exploited.
3. Vaccine BCG is used.
4. The immunization of volunteers is employed.

**Key Points**

1. Development of vaccination became such event, which changed the fate of human civilization in total and the fate of a single human being. The modern vaccines counteract both infections and cancer, and they will prevent and even cure the other types of immunopathology in the near future.
2. Four types of subunit vaccines against a wide variety of infections have already been developed and manufactured, whereas recombinant vector, DNA, dendritic cell-based, and other types of genetically engineered vaccines have been developing.

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# Immune Enhancement Therapy

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**Electronic supplementary material** The online version of this chapter ([https://doi.org/10.1007/978-3-030-03323-1\\_9](https://doi.org/10.1007/978-3-030-03323-1_9)) contains supplementary material, which is available to authorized users.

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## Learning Objectives

*Knowledge.* Upon successful completion of the chapter, students should be able to:

1. Know the immune enhancement therapy as a form of interventional immunology.
2. Describe the mechanisms of immune enhancement therapy.
3. List the groups of immune enhancement medications and describe separate medications

*Acquired Skills.* Upon successful completion of the chapter, students should demonstrate the following skills, including:

1. Interpret the knowledge related to immune enhancement therapy.
2. Critically evaluate the clinical literature about immune enhancement therapy.
3. Discuss the scientific articles from the current research literature to criticize new development of novel approaches in immune enhancement therapy.
4. Obtain a patient's history including history of present illness; past medical history; social, family, and occupational history; and review of systems.
5. Perform a patient's physical examination in a thorough manner.
6. Explain the rationale for choice of the interventional immunology in a patient.
7. Attain a clear perception of the presented immunology definitions expressed orally and in written form.
8. Formulate the introduced immunology terms.
9. Correctly answer quiz questions.

*Attitude and Professional Behaviors.* Students should be able to:

1. Have the readiness to be hardworking.
2. Behave professionally at all times.
3. Recognize the importance of studying and demonstrate a commitment.
4. Demonstrate the consideration of the patient's feelings, ethnic, religious, cultural, and social background and be able to display empathy.

## 9.1 Introduction

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The reader can find the information on medications used for the immune enhancement therapy for immunocompromised conditions, which take place in the pathology of a wide array of involved organs and tissues of the human body and must be a useful tool for practitioners. It should be noted this critical topic is very rare in immunology manuals.

### Definitions

- *Interventional immunology* is the complex of therapeutic approaches intended for the treatment for specific forms of immunopathology.
- *Immune enhancement therapy* is a type of interventional immunology that upregulates the weakened immune system in patients of immunocompromised conditions.

Immune enhancement therapy is a part of the interventional immunology, which also includes the use of (1) immunosuppressive medications such as blockers of signaling molecules, costimulatory molecules, cytokines, chemokines, receptors, and corticosteroids and antimetabolites, (2) anti-allergy drugs against allergic inflammation (e.g., antihistamines, anti-leukotrienes, etc.), and (3) allergen-specific immunotherapy (ASIT).

The objective of *immune enhancement therapy* is to activate the immune responses against antigens, which are not well-recognized, and potentiate mechanisms of the innate immune system. Patients who died of sepsis compared to patients who died of nonseptic conditions have immunological laboratory data consistent with immunosuppression. Many diseases may, directly or indirectly, be traced back to a disorder of the immune system. Suppression of the immune system induces significant changes in the body. *Secondary immunocompromised conditions* have been shown to be caused by some viruses, bacteria, fungi, and protozoans; various external stresses, including pesticides; alcohol and tobacco abuse; antibiotics; chemotherapy; birth control pills; corticosteroids; other drug therapies; etc. Thus, immune enhancement therapy may be an important approach in these similar conditions.

Immune enhancement medications may be categorized by (1) origin (organs of the immune system, microbes themselves, synthetic, or recombinant drugs) and (2) point of application (T cells, B cells, phagocytes, etc.). Routes of administration of the drugs may be different including subcutaneous, intramuscular, oral, sublingual, rectal, topical, etc.

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Mechanisms of action of immune enhancement medications include:

1. Vaccine-like action
2. "Pattern"-like effect
3. Activation of separate cell functions and processes in the immune system
4. Replacement action (e.g., immunoglobulins, cytokines, etc.)
5. Anti-inflammatory effect

**From a clinical viewpoint** because of empirical approach with immune enhancement therapy, there has been some controversy in the interpretation of indications to prescribe it. Some conditions may have underlying causes, which are not based on a malfunctioning immune system.

## 9.2 Products of the Thymus and Spleen

To date, more than 20 thymus peptides have been isolated from the cattle's whole thymus extract. Five of them have already been investigated in depth. Throughout the years, three major areas of indication have taken shape due to the dominant regulatory ability of the thymus peptides: postoperative tumor care, chronic recurrent infections, and autoimmune disorders.

### 9.2.1 TFX-Thymomodulin®

**Composition** TFX-Thymomodulin® (see ■ Fig. 9.1) is a family of six peptides, biotechnologically derived from the thymus glands of young calves.

■ Fig. 9.1 TFX-Thymomodulin



**Mechanisms of action** The drug works as a substitute for the physiological functions of the thymus. With T-cell deficiency, TFX-Thymomodulin® recruits immature immune system cells in the bone marrow and stimulates their maturation to the fully active T-cell phase in the lymphatic organs. TFX-Thymomodulin® increases granulopoiesis and erythropoiesis by acting on the bone marrow. It can be used for all diseases with primary and secondary immune system disturbances involving T cells, which are thymus dependent, and for a wide range of symptoms such as chronic viral, bacterial, and fungal infections, allergic and autoimmune reactions, and certain lymphoproliferative syndromes.

**Indications** All systemic diseases of the connective tissue, nervous tissue, muscles, vessels, and the internal organs caused by the deficient immune function of the thymus: chronic virus hepatitis, polyneuropathy, encephalopathy, autoimmune spinal cord diseases, acute dermatomyositis, allergic skin diseases, SLE, hypo- and hypergammaglobulinemias, and cutaneous and organ-involved mycoses.

**Contraindications** No absolute contraindications are known. Relative contraindications include pregnancy, menstruation, certain endocrinological syndromes, and the period of physiological activity of the thymus up through the time of sexual maturation.

**Side effects** No side effects are known. At the injection site, erythema and/or slight sensitivity may develop. If this occurs, a 1- or 2-day break in the treatment is indicated.

**Dosage** The dosage needs to be determined on an individual basis depending on the health of the patient. The following dosage is recommended: 10–20 mg/day for 30 days followed by 20–50 mg/week, intramuscular. The continued administration, dosage, and duration of the treatment depend on the therapeutic effect and immunological results.

### 9.2.2 Thymex-L®

**Composition** Each vial of Thymex-L® (see ■ Fig. 9.2) contains 150 mg lyophilized, sterile, total thymus extract – THX by Dr. Pesic – isolated from the fresh juvenile calf thymus and

■ Fig. 9.2 Thymex-L



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standardized to 0.6 mg/mg thymus cytoplasmatic protein. Besides immune effective peptides and proteins, the total thymus extract is composed of adenosine deaminase, purine nucleoside phosphorylase, superoxide dismutase, glutathione reductase, glutathione-S-transferase, glutathione peroxidase, and peptidase activities.

**Indications** Illnesses due to reduced immunity, especially decreased T-cell immunity, rheumatoid arthritis, arthrosis, microcellular lung cancer, and geriatrics. For treatment of enzymopathies related to adenosine deaminase, purine nucleoside phosphorylases, and certain peptidases. Thymex-L<sup>®</sup> therapy has yielded good results in adjuvant treatment for some types of cancer (breast, lungs, uterus, and prostate) as well as Hodgkin's lymphoma and Kaposi's sarcoma.

The prophylactic role of Thymex-L<sup>®</sup> against the abnormal development of cellular tissue is significant.

**Contraindications** Internal hemorrhage, long-term therapy with cortisone as well as cortisone derivatives, and other hormones of the suprarenal gland. After completion of cortisone treatment, Thymex-L<sup>®</sup> therapy should only be started after a 6-week-waiting period. Thymex-L<sup>®</sup> should not be given during the first 3 months of pregnancy.

**Side effects** After the first injection, it is possible that slight local itching, redness, or swelling could develop. It should be treated with ointments containing antihistaminic substances or heparin. In some cases, there may be a temperature and/or fever. It is recommended to use antihistaminic per os or discontinue treatment for a day or 2-day break. No strong allergic reactions or anaphylaxis has been observed.

**Dosage** Unless otherwise prescribed, intramuscular or subcutaneous injections are to be administered every other day for a total of 10–20 injections depending on the patient's

condition and the stage of the illness. The treatment can be repeated after an interval of 3–12 months. If necessary, long-term treatment with Thymex-L® can be administered. Then, one injection twice a week is recommended.

### 9.2.3 Splenin®

**Composition** Each vial of Splenin® (see ■ Fig. 9.3) contains 50 mg of polypeptides from porcine spleen as a freeze-dried powder as the active ingredient. Inert ingredients – none.

**Mechanisms of action** Splenin® is an immunomodulator, immunocorrector, and immunoregenerator.

**Indications** Splenin® is indicated for all immune deficiency symptoms, chronic and degenerative diseases, wounds that do not heal, vascular diseases, erection dysfunctions in males, menopausal symptoms in females, malignant growths after the operation, radiation therapy, and chemotherapy.

**Contraindications** Splenin® should not be administered when there is a known allergy to the porcine spleen. There is no information about its use during pregnancy and nursing.

**Side effects** In single cases, an allergic exanthema may occur. In rare cases, especially in patients predisposed to allergies, reactions ranging from a hypersensitive reaction to anaphylactic shock could occur.



■ Fig. 9.3 Splenin

**Dosage** One vial of Splenin<sup>®</sup> administered daily, for 1 week from Monday to Friday, then three times a week, for 2 weeks. Subsequently, one vial of Splenin<sup>®</sup> is administered twice a week, for 3 weeks. Further administration of Splenin<sup>®</sup> is determined on an individual case basis by the family practitioner and depends on the patient's condition and tolerance to therapy. Repeated treatment is recommended after 6 weeks or 3 months. In severe cases, long-term therapy may be considered when one vial of Splenin<sup>®</sup> is administered 1–2 times a week.

## 9.3 Immunoglobulin Therapy

Intravenous immunoglobulins (IVIG) pooled from the plasma of approximately a thousand or more blood donors contain more than 95% unmodified IgG, which has intact Fc-dependent effector functions, and only trace amounts of IgA and IgM. IVIG is an immunomodulating medication that has multiple activities including antibody replacement, neutralization of microbial toxins, modulation of complement activation and phagocytic activity, suppression of idiotypic antibodies, saturation of Fc receptors on macrophages, amplification of  $\beta$ -lactam antibiotics, and inhibition of a variety of pro-inflammatory mediators (e.g., cytokines, chemokines, and metalloproteinases).

There are, at least, three problems, which may be associated with IVIG, as follows:

- Life-threatening *HIV*, *hepatitis B virus*, *hepatitis C virus*, and human parvovirus B19 may be transmitted by IVIG administration.
- Anti-IgA autoantibodies may be constituted during IVIG administration.
- IVIG may cause severe systemic anaphylactic reactions.

However, new-generation IVIG medications provide a high level of antiviral safety and unmodified IVIG molecules, which reduce cases of viral contamination and allergic reactions.

### 9.3.1 Octagam<sup>®</sup> (Human Immune Globulin G)

**Composition** Each bottle of Octagam<sup>®</sup> (see ■ Fig. 9.4) contains plasma protein 50 mg/mL including at least 95% of human IgG, maltose, tributyl phosphate, octoxynol, and water.

**Mechanisms of action** Antibody replacement action in humoral immunodeficiencies and immunomodulating effects in a variety of diseases of the immune system.

**Indications** Replacement therapy is performed in primary humoral immunodeficiency diseases, multiple myeloma, chronic lymphoblastic leukemia with severe secondary hypogammaglobulinemia and recurrent infections, and congenital *HIV* in babies and toddlers. Immunomodulating therapy is performed in idiopathic thrombocytopenia purpura, Guillain-Barré syndrome, Kawasaki disease, and bone marrow transplantation.

**Contraindications** IgA deficiencies, maltose intolerance, planned vaccinations, and individual acute severe hypersensitivity reactions to human immunoglobulin. In pregnancy and breastfeeding, the risks and benefits of the treatment must be discussed.

■ Fig. 9.4 Octagam



**Side effects** Flushing, headache, dizziness, chills, muscle cramps, back/joint pain, fever, nausea, vomiting, easy bleeding/bruising, fainting, fast/irregular heartbeat, or unusual tiredness may occur. Pain, redness, and swelling at the injection site may also occur. A very serious allergic reaction to this drug such as trouble breathing, swelling of throat, angioedema, or anaphylaxis is rare. Noncardiogenic pulmonary edema, hemolysis, thrombotic events, acute renal failure, and aseptic meningitis syndrome may rarely occur during the treatment and afterward.

**Dosage** For replacement therapy in primary humoral immunodeficiency diseases, the dose of Octagam® 5% liquid is 300–600 mg/kg body weight (6–12 mL/kg) administered intravenously every 3–4 weeks. The dosage may be adjusted over time to achieve the desired concentration and clinical responses. If a patient on regular treatment misses a dose, the missed dose should be administered as soon as possible, and then treatment should continue as previously. Infusion rates: 0.5 mg/kg/min (30 mg/kg/h for the first 30 min; if tolerated, advance to 1 mg/kg/min (60 mg/kg/h) for the second 30 min; and if further tolerated, advance to 2 mg/kg/min (120 mg/kg/h) for the third 30 min. After that, the infusion can be maintained at a rate up to, but not exceeding, 3.33 mg/kg/min (200 mg/kg/h).

## 9.4 Recombinant Cytokines

Recombinant ILs, CSFs, and IFNs are widely employed in immune disorders, malignancies, chronic recurrent infections, and complications related to chemotherapy in graft transplantation.

### 9.4.1 Proleukin® (Aldesleukin, a Human Recombinant Interleukin-2 Product)

**Composition** Each vial of Proleukin® (see ■ Fig. 9.5) contains 22 million IU (international units) of aldesleukin as a lyophilized (freeze-dried) powder, with diluent.

**Mechanisms of action** Aldesleukin is a recombinant protein that has the same action as native human IL2. The IL2 is secreted by T cells, upregulates T-cell and B-cell growth in the course of adaptive immune responses, and potentiates CD8 + T cells and NK cells to fight cancer.

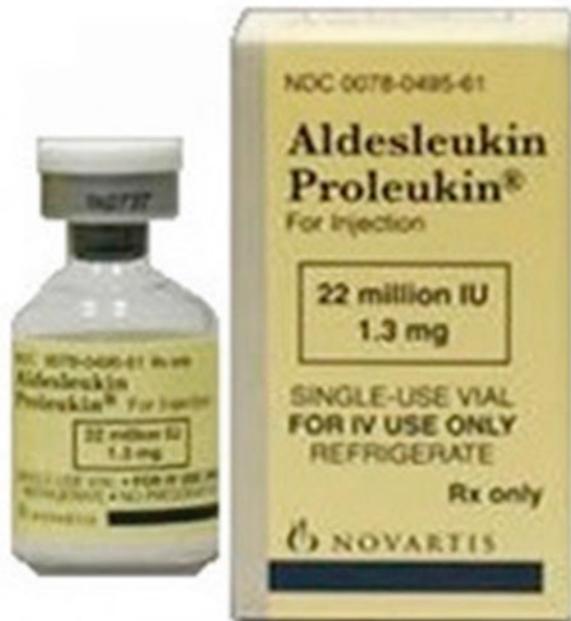
**Indications** Widespread (metastatic) cancer of the kidney, skin melanoma, acute myeloblastic leukemia, non-Hodgkin's lymphoma, *HIV* infection, Kaposi's sarcoma, and leprosy.

**Contraindications** Aldesleukin causes side effects in almost every organ. According to a variety of side effects, often severe and dangerous, aldesleukin cannot be given to patients who are physically and mentally intolerant to it. It is not known whether aldesleukin can cause harm to the fetus.

**Side effects** This medication can cause capillary leak syndrome (CLS), a serious condition that can sometimes be fatal. CLS is characterized by swelling, severe dizziness, fainting, irregular heartbeat, chest pain (angina), trouble breathing, change in the amount of urine, mental/mood changes, severe stomach/abdominal pain, and black stools. Problems associated with capillary leak syndrome may also include congestion

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■ Fig. 9.5 Aldesleukin



in the lungs, difficulty breathing, wheezing, and respiratory failure. Bleeding from the stomach and intestines, nausea, vomiting, diarrhea, constipation, generalized pain, kidney or liver damage, loss of consciousness, paranoia, hallucinations, drowsiness, visual changes, alterations, loss of taste sensation, sleep disturbances, headache, fatigue, weakness, malaise, loss of appetite, hypothyroidism, anemia, leukopenia, thrombocytopenia, itching, angioedema, rashes, and infection also can occur. Most of the side effects caused by capillary leak syndrome begin to resolve a few hours after stopping aldesleukin therapy.

**Dosage** The recommended dose is 600,000 IU/kg intravenously over 15 min every 8 h for 14 doses followed by 9 days of rest then another 14 doses.

### 9.4.2 Viferon® (A Human Recombinant Interferon $\alpha$ 2b Product)

**Composition** Each rectal suppository of Viferon® (see  Fig. 9.6) contains human recombinant interferon  $\alpha$ 2b, ascorbic acid,  $\alpha$ -tocopherol acetate, and cocoa butter. There are rectal suppositories of 150,000 IU, 500,000 IU, 1,000,000 IU, and 3,000,000 IU.



 Fig. 9.6 Viferon

**Mechanisms of action** Viferon® is a recombinant protein that has the same action as native human IFN $\alpha$ 2b. The IFN $\alpha$  is produced by leukocytes and other cell types and takes part in the innate immune system as a potent antiviral agent to promote the cytostasis of target cells. IFN-inducible enzymes inside cells infected by virus individually block viral transcription, degrade viral RNA, inhibit translation, and control all steps of viral replication. IFN $\alpha$  is an anti-inflammatory cytokine and stimulator of NK-cell and macrophage activity. As highly active antioxidants, ascorbic acid and  $\alpha$ -tocopherol acetate have anti-inflammatory, membrane stabilizing, and regenerating effects.

**Indications** (1) Acute respiratory viral infections including influenza in children and adults, as a part of complex therapy; (2) infectious inflammatory diseases in newborns including premature newborns, such as meningitis (viral and bacterial), sepsis, and intra-uterine infections, as a part of complex therapy; (3) chronic viral hepatitis B and chronic viral hepatitis C in children and adults, as a part of complex therapy; and (4) infectious inflammatory genitourinary diseases including *herpes simplex virus (HSV)* infection in adults, as a part of complex therapy.

**Contraindications** Hypersensitivity to any component of the product.

Lactation and pregnancy: the product is approved for use from the 14th week of gestational age; use of the product during breastfeeding is not restricted.

**Side effects** Rare allergic reactions (rash and itching). These events are reversible and disappear 72 h after treatment discontinuation.

### Dosage

1. In acute respiratory viral infections, the recommended dose of Viferon® 500,000 IU for adults and children aged 7 and older is 1 suppository two times a day every 12 h daily, over 5 days; based on clinical indications, treatment may be prolonged.
2. In the pathology of newborns and premature newborns (gestational age over 34 weeks), the recommended dose of Viferon® 150,000 IU is 1 suppository two times a day every 12 h daily, over 5 days; based on clinical indications, treatment may be prolonged; the interval between courses is 5 days.
3. In chronic hepatitis B and chronic hepatitis C, the recommended dose of Viferon® 3,000,000 IU in adult patients is 1 suppository two times a day every 12 h daily, over 10 days, then three times a week every other day, over 6–12 months; for children aged 6 months and younger, it is 300,000–500,000 IU a day, and for children aged 6–12 months, 500,000 IU a day.
4. In HSV skin and mucosal infection including genital HSV infection, and other infectious, inflammatory genitourinary diseases, the recommended dose of Viferon® 1,000,000 IU for adults is 1 suppository two times a day every 12 h daily, over 10 days and longer if a case of relapse occurs; based on clinical indications, treatment may be prolonged.

## 9.5 Synthetic Products

### 9.5.1 Ampligen® (Rintatolimod)

**Composition** Each bottle of Ampligen® (see ■ Fig. 9.7) contains 200 mg of rintatolimod.

**Mechanisms of action** Ampligen® may stimulate the innate immune system to eradicate pathogens by activating the production of an enzyme called RNase L. This enzyme along with another enzyme, 2',5'-oligoadenylate-synthetase, degrades all RNA in cells to inhibit translation upon viral replication. Accumulation of an inactive form of RNase L may be associated with chronic fatigue syndrome (CFS). In addition, Ampligen® is an agonist of TLR3.

**Indications** CFS; ovarian, peritoneal, and colorectal cancers; HIV/AIDS; swine and avian flu; and ebola virus infections.

**Contraindications** Ampligen® cannot be given to patients who are physically and mentally intolerant to it. It is not known whether Ampligen® can cause harm to the fetus.



■ Fig. 9.7 Ampligen

**Side effects** Mild flushing, tightness of the chest, rapid heartbeat, low blood pressure, anxiety, shortness of breath, dizziness, feeling hot, sweating, nausea, liver enzyme level changes, diarrhea, itching, rash, leukopenia, and confusion.

**Dosage** The recommended dose of Ampligen® is 400 mg intravenously twice a week, for 12–18 months.

### 9.5.2 Cycloferon® (Meglumine Acridonacetate)

**Composition** Each ampoule of Cycloferon® (see ■ Fig. 9.8) contains 250 mg of meglumine acridonacetate. Excipients: water for injection to 2.0 mL.

**Mechanisms of action** Meglumine acridonacetate is an inducer of the formation of endogenous IFN $\alpha$  and activator of NK cells. The effectiveness of the drug is associated with a broad-spectrum of biological activity such as anti-inflammatory, antiproliferative, antitumor, antiviral, and immunomodulatory effects.

**Indications** Chronic recurrent viral infections, neuroinfections, rheumatic and systemic connective tissue diseases, and degenerative diseases of the joints, as a part of complex therapy.

**Contraindications** Pregnancy, lactation, allergic reactions, decompensated cirrhosis of the liver, and children under 4 years of age.

**Side effects** Allergic manifestations.

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■ Fig. 9.8 Cycloferon



**Dosage** In adults, meglumine acridonacetate 250 mg is given intramuscularly or intravenously one time every 2 days, over 20 days. The recommended dosage in children is 6–10 mg/kg body weight.

### 9.5.3 Polyoxidonium® (Azoximer Bromide)

**Composition** Polyoxidonium® (see ■ Fig. 9.9) is manufactured as pills containing 12 mg, rectal suppositories containing 6 and 12 mg, and bottles containing 3 and 6 mg of sterile lyophilized powder.

**Mechanisms of action** Azoximer bromide is a macromolecular compound, which acts as an immunomodulator, detoxifier, and antioxidant. Azoximer bromide activates circulating B cells, phagocytes, and tissue macrophages, which contribute to the more rapid elimination of the pathogens from the body. The medication can rid the body of toxins and heavy metals and inhibit lipid peroxidation.

**Indications** Acute and chronic recurrent infectious and inflammatory diseases of the respiratory organs and genitourinary organs; allergic disease complicated by recurrent bacterial, fungal, and viral infections (including atopic dermatitis, allergic rhinitis, and bronchial asthma); postoperative purulent complications; septic states; and immunocompromised conditions after chemotherapy and radiation therapy.

**Contraindications** Acute renal failure, individual intolerance, acute allergic reactions, pregnancy, and lactation.

**Side effects** Pain and redness at the injection site may occur.

**Dosage** The recommended dose of azoximer bromide in pills for adults is 12 mg two times a day before meals, over 10–15 days, whereas for children over 12 years, the dose is 12 mg daily; based on clinical indications, treatment may be prolonged.

■ Fig. 9.9 Polyoxidonium



The recommended dose of azoximer bromide in suppositories for adults is 12 mg daily over 3 days, then once every 2–3 days, over 20–24 days, whereas for children over 6 years, the dose is 6 mg two times a week; based on clinical indications, treatment may be prolonged.

The recommended dose of azoximer bromide in bottles for adults is 6 mg daily intramuscular or intravenously (on a drip) during 3 days, then once every 2 days, over 10–15 days, whereas for children over 6 months, the dose is 3 mg once every 2 days or two times a week; the lyophilized powder is diluted by 1.5–2 mL of water for injection or 0.9% solution of sodium chloride; based on clinical indications, treatment may be prolonged.

## 9.6 Mucosal Autovaccines

Mucosal autovaccines are bacterial lysates used as oral or sublingual immunostimulants. The principle of their action is to potentiate the innate immunity and adaptive immune responses at the mucosal level to prevent and help fight infections. There are two different methods to lyse opportunistic bacteria for the mucosal vaccine preparation, chemical and mechanical. As contrasted to the chemical method, the mechanical approach allows preserving a higher immunogenicity potential of such bacterial lysates.

### 9

#### 9.6.1 Ismigen<sup>®</sup>, Immubron<sup>®</sup> (Bacterial Lysates Mixture)

**Composition** One tablet of Ismigen<sup>®</sup> and Immubron<sup>®</sup> (see ■ Fig. 9.10) contains lyophilized bacterial lysates 50 mg, of which 7 mg is corresponding to *Staphylococcus aureus* 6 billion, *Streptococcus pyogenes* 6 billion, *Streptococcus viridans* 6 billion, *Klebsiella pneumoniae* 6 billion, *Klebsiella ozaenae* 6 billion, *Haemophilus influenzae type b* 6 billion, *Neisseria catarrhalis* 6 billion, and *Diplococcus pneumoniae* 6 billion. Excipients: silicon dioxide, microcrystalline cellulose, calcium phosphate dibasic, magnesium stearate, ammonium glycyrrhizinate, and the essence of mint powder.

**Mechanisms of action** These products stimulate an advanced B-cell-mediated response and the innate immune system at the mucosal level (the respiratory tract) exhibiting vaccine-like and “pattern”-like effects.



■ Fig. 9.10 Ismigen/Immubron

**Indications** Recurrent infections of the upper and lower respiratory tract, prevention of recurrent respiratory infections, and addition to allergen-specific immunotherapy (ASIT).

**Contraindications** Children under 3 years of age, hypersensitivity, pregnancy, and lactation.

**Side effects** Nausea, abdominal pain, rash, allergic reactions, dyspnea, fever, and coughing.

**Dosage** One tablet 7 mg is used regardless of patient age, daily sublingually, before the first meal, over 10 days in three consecutive months. If a patient on regular treatment misses a dose, the missed dose should not be administered. The course may be repeated one more time during a year.

### 9.6.2 Ribomunyl® (Bacterial Lysate Mixture)

**Composition** Ribomunyl® (see ■ Fig. 9.11) is manufactured as tablets 0.25 and 0.75 mg and granules 0.75 mg. Ribomunyl® consists of ribosomal antigens of *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Streptococcus viridans* and membrane proteoglycans of *Klebsiella pneumoniae*. Excipients: polyvidon, D-mannitol, silicon dioxide, magnesium stearate, and sorbitol.

**Mechanisms of action** This product stimulates an advanced B-cell-mediated response and the innate immune system at the mucosal level (the respiratory tract) exhibiting vaccine-like and “pattern”-like effects.

■ Fig. 9.11 Ribomunyl



**Indications** Recurrent infections of the upper and lower respiratory tract, prevention of recurrent respiratory infections, and addition to allergen-specific immunotherapy (ASIT).

**Contraindications** Children under 6 months of age, hypersensitivity, autoimmune diseases, pregnancy, and lactation.

**Side effects** Ribomunil® does not cause serious side effects, but sometimes nausea, vomiting, diarrhea, increased salivation, urticaria, or angioedema may occur; these symptoms do not require discontinuation of the medication (except for allergic reactions).

**Dosage** In the first 3 weeks of treatment (see ■ Fig. 9.12), Ribomunyl® 0.75 mg is administered daily regardless of patient age, before the first meal, for the first 4 days of each week, and then for the first 4 days of the month for 2–5 consecutive months. Granules of 0.75 are usually given to children as a boiled water solution. If a patient on regular treatment misses a dose, the missed dose should not be administered.

### 9.6.3 Uro-Vaxom® (Bacterial Lysates)

Composition: Each capsule of Uro-Vaxom® (see ■ Fig. 9.13) contains 6 mg of lyophilized bacterial lysates of *Escherichia coli*. Excipients: pregelatinized maize starch, magnesium silicate, magnesium stearate, propyl gallate (E 310), sodium glutamate, mannitol, gelatine, ferric oxides, and titanium dioxide.

**Mechanisms of action** Uro-Vaxom® stimulates the innate immune system at the mucosal level (genitourinary tract) exhibiting “pattern”-like effect. In addition, this medication induces production of polyspecific sIgA in the urine.

**Indications** Prevention of recurrent lower genitourinary tract infections, as a part of complex therapy for acute genitourinary tract infections.

1st month							2nd-5th months						
1	2	3	4	5	6	7	1	2	3	4	5	6	7
8	9	10	11	12	13	14	8	9	10	11	12	13	14
15	16	17	18	19	20	21	15	16	17	18	19	20	21
22	23	24	25	26	27	28	22	23	24	25	26	27	28
29	30	31					29	30	31				

■ Fig. 9.12 Drug regimen for ribomunyl



■ Fig. 9.13 Uro-Vaxom

**Contraindications** Children under 4 years of age, hypersensitivity, pregnancy, and lactation.

**Side effects** Diarrhea, nausea, abdominal pain, itching, rash, and slight fever may rarely occur. In cases of skin reactions or fever, the treatment should be interrupted as these may constitute more severe side effects.

**Dosage** One capsule 6 mg is used regardless of patient age daily, on an empty stomach, over 10 days for three consecutive months. Based on clinical indications, treatment may be carried out daily for three consecutive months. If a patient on regular treatment misses a dose, the missed dose should not be administered.

#### 9.6.4 SymbioLact<sup>®</sup> Compositum

**Composition** One sachet of SymbioLact<sup>®</sup> Compositum (see ■ Fig. 9.14) contains 2 g of which *Lactobacillus acidophilus*, 25 mg; *Lactobacillus casei*, 25 mg; *Lactobacillus lactis*, 25 mg; *Bifidobacterium bifidum*, 12.5 mg, *Bifidobacterium lactis*, 12.5 mg, and *Lactobacillus salivarius*, 2.6 mg. Additional components: maltodextrin, maize starch, silicon dioxide, and biotin.

**Mechanisms of action** SymbioLact<sup>®</sup> Compositum plays a significant nutritional physiological role in defense against harmful microbes, production of essential vitamins, maintenance of the intestinal functions, and support of the mucosal immune system.

**Indications** As a food supplement, in intestinal dysbioses and during the administration of antibiotics.

**Contraindications** Individual intolerance and permanent intravascular catheter.

**Side effects** Rare dyspeptic disorders.

**Dosage** For adults, the recommended dosage is the contents of one sachet dissolved in a glass of water (100 mL) 1–2 times daily during meals. For babies and small children, the



■ Fig. 9.14 SymbioLact Compositum

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recommended dosage is the content of one sachet stirred into lukewarm tea, baby food, or similar once a day.

## 9.7 Immune Enhancement Metabolites

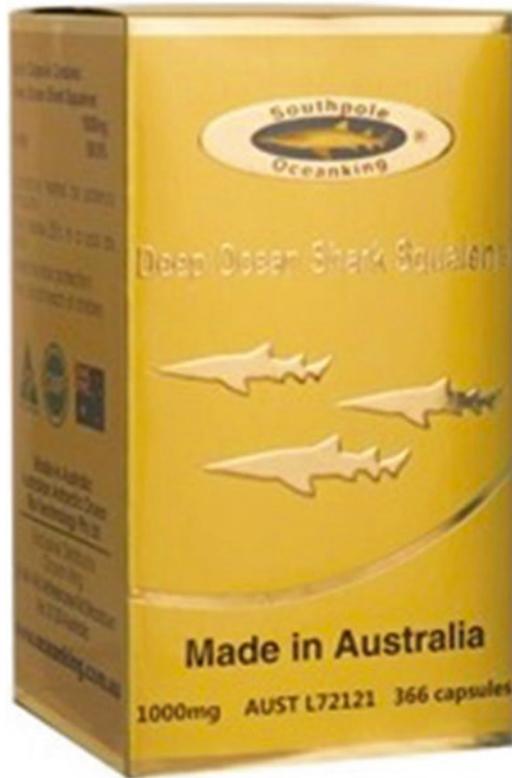
Vitamins, trace elements, and some other substances if deficient in the body may lead to both metabolic and immune disorders. It is revealed coilecalciferol (or vitamin D<sub>3</sub>) and other vitamins, squalene, copper, zinc, selenium, etc. are very important for both innate and adaptive immunity. Nutritional and endogenous deficiency of these metabolites results in acquired immunocompromised conditions and diminishes outcomes of vaccination.

### 9.7.1 Squalene

**Composition** Squalene is low-density hydrocarbon, a precursor for the synthesis of cholesterol and steroid hormones in the body. Each capsule contains 1000 mg squalene from the liver of deep ocean sharks (see ■ Fig. 9.15). Squalene is produced by the extraction from the shark's liver and vegetable sources such as argan oil.

**Mechanisms of action** The precise action mechanism of squalene remains unclear, but its adjuvant effect has been well characterized. Squalene may exert antitoxic, antioxidant, and ant clotting effects and play a key role in the prevention of recurrent respiratory infections.

**Indications** As a component of some vaccines, cosmetic use, prevention of recurrent respiratory infections, and thrombosis.



■ Fig. 9.15 Squalene

**Contraindications** Individual intolerance and predisposition to hemorrhagic conditions.

**Side effects** Infrequent.

**Dosage** 1000–3000 mg preferably with meals daily for 1 year as a dietary supplement.

■ **Quiz**

Reading a question, please choose only one right answer.

❓ **Question 1**

The immune enhancement therapy may be used in:

1. Liver transplantation.
2. Secondary immunocompromised conditions.
3. Any graft transplantation.
4. Common flu.

**? Question 2**

The immune enhancement medications do not exert mechanisms of action:

1. Vaccine-like action.
2. Anti-inflammatory effect.
3. Immunosuppressive effect.
4. "Pattern"-like action.

**? Question 3**

TFX-Thymomodulin<sup>®</sup> is a product made of:

1. The spleen.
2. The lymph nodes.
3. The bone marrow.
4. The thymus.

**? Question 4**

Splenin<sup>®</sup> is a product made of:

1. The thymus.
2. The spleen.
3. The appendix.
4. The lymph nodes.

**? Question 5**

A problem, which may be linked to intravenous immunoglobulins (IVIG):

1. Breakdown of self-tolerance.
2. Production of anti-IgA autoantibodies.
3. Pro-inflammatory effect.
4. "Pattern"-like effect.

**? Question 6**

One more problem, which may be linked to IVIG:

1. Pro-inflammatory effect.
2. Breakdown of self-tolerance.
3. Transmission of life-threatening viruses.
4. Overactivation of the immune system.

**? Question 7**

Action mechanism of the IVIG is:

1. Vaccine-like action.
2. "Pattern"-like action.
3. Replacement action.
4. Activation of separate cell functions.

**? Question 8**

Proleukin<sup>®</sup> (aldesleukin) contains:

1. Recombinant IL1.
2. Staphylococcal toxoid.
3. Recombinant IFN $\gamma$ .
4. Recombinant IL2.

**? Question 9**

Viferon<sup>®</sup> contains:

1. Recombinant IFN $\gamma$ .
2. Recombinant IL2.
3. Recombinant IFN $\alpha$ .
4. Virus-like particle (VLP).

**? Question 10**

Action mechanism of the viferon<sup>®</sup> is:

1. Activation of separate cell functions.
2. "Pattern"-like action.
3. Replacement action.
4. Vaccine-like action.

**? Question 11**

Cycloferon<sup>®</sup> (meglumine acridonacetate) is:

1. An immunoglobulin.
2. An inducer of the formation of endogenous IFN $\alpha$  and activator of NK cells.
3. A mucosal vaccine.
4. Recombinant IFN $\alpha$ .

**? Question 12**

Mucosal autovaccines are:

1. Bacterial lysates.
2. Recombinant cytokines.
3. Thymic products.
4. Recombinant interferons.

**? Question 13**

Ismigen<sup>®</sup> and Imubron<sup>®</sup> are made by:

1. Chemical method of bacterial lysis.
2. Genetic engineering.
3. Mechanical method of bacterial lysis.
4. "Passage" attenuation.

**? Question 14**

Ismigen<sup>®</sup> and Immubron<sup>®</sup> are used in:

1. Sepsis.
2. Recurrent infections of the respiratory tract.
3. Recurrent infections of the gastrointestinal tract.
4. Recurrent infections of the genitourinary tract.

**? Question 15**

Uro-Vaxom<sup>®</sup> is used in:

1. Recurrent infections of the genitourinary tract.
2. Pneumonia.
3. Recurrent infections of the respiratory tract.
4. Sepsis.

**? Question 16**

Polyoxidonium<sup>®</sup> (azoximer bromide) is:

1. A mucosal vaccine.
2. A symbiotic.
3. A thymic product.
4. A synthetic product.

**Key Points**

1. Interventional immunology is the complex of therapeutic approaches intended for the treatment for specific forms of immunopathology. The interventional immunology includes immune enhancement therapy, immunosuppressive therapy, anti-allergy drug treatment, and allergen-specific immunotherapy (ASIT). Immunostimulants are used in a variety of immunocompromised conditions such as recurrent respiratory infections, skin pyogenic infections, bacterial vaginosis, recurrent genitourinary tract infections, complicated chemotherapy, etc.
2. Immune enhancement therapy includes the use of products made of the thymus and spleen, intravenous human immunoglobulins, recombinant anti-inflammatory cytokines and interferons, synthetic compounds, mucosal autovaccines, and natural metabolites.

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# Anti-allergy Medications

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**Electronic supplementary material** The online version of this chapter ([https://doi.org/10.1007/978-3-030-03323-1\\_10](https://doi.org/10.1007/978-3-030-03323-1_10)) contains supplementary material, which is available to authorized users.

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## Learning Objectives

*Knowledge.* Upon successful completion of the chapter, students should be able to:

1. Distinguish between two modalities used in treatment for patients with allergic conditions.
2. Describe antihistamines.
3. Be familiar with the membrane stabilizers.
4. Describe topical corticosteroids.
5. Be familiar with the use of monoclonal antibodies in treatment for allergic conditions.

*Acquired Skills.* Upon successful completion of the chapter, students should demonstrate the following skills, including:

1. Interpret the knowledge related to anti-allergy medications.
2. Critically evaluate the clinical literature about medication therapy for allergic disorders.
3. Discuss the scientific articles from the current research literature to criticize experimental and clinical data and formulation of new hypotheses in allergy.
4. Obtain a patient's history including history of present illness; past medical history; social, family, and occupational history; and review of systems.
5. Perform a patient's physical examination in a thorough manner.
6. Explain the rationale for choice of the therapy for allergic conditions.
7. Attain a clear perception of the presented immunology definitions expressed orally and in written form.
8. Formulate the presented immunology terms.
9. Correctly answer quiz questions.

*Attitude and Professional Behaviors.* Students should be able to:

1. Have the readiness to be hardworking.
2. Behave professionally at all times.
3. Recognize the importance of studying and demonstrate a commitment.
4. Demonstrate the consideration of the patient's feelings, ethnic, religious, cultural, and social background and be able to display empathy.

# 10

## 10.1 Introduction

---

There is the description of medications, which use in the protocol treatment for allergy. Two main modalities of treatment for allergic conditions are discussed.

### Definitions

*Anti-allergy therapy* is a part of the interventional immunology, which includes the use of medications directed against acute and chronic allergic inflammation.

**From a clinical viewpoint,** the purpose of anti-allergy therapy for allergic diseases and syndromes is the prevention of emergency department visits and hospitalizations, decrease in allergic signs and symptoms, achievement of long-term control of these common chronic conditions, and quality of life maintenance.

There are two treatment modalities, which may be used only for individuals with atopic allergic conditions:

1. Medication therapy
2. Allergen-specific immunotherapy (ASIT), commonly known as allergy shots

Medication therapy includes a number of various drugs with different mechanisms of action.

## 10.2 Antihistamines

The 1957 Nobel Laureate, D. Bovet, discovered *antihistamines* in 1937 (see ■ Fig. 10.1), which block the neurotransmitter histamine and widely exploit in allergy therapy. Antihistamines are probably the best known type of allergy remedies, and most are readily available from a pharmacy without the prescription.

Antihistamines act on the tissues through histamine receptors. There are four types of histamine receptors (see ■ Table 10.1).

Antihistamines, H1 blockers, compete with histamine for H1 receptors, and as a result, this diminishes histamine's effects. They act best when taken before exposure to the allergen and may be used both at the systemic and topical levels.

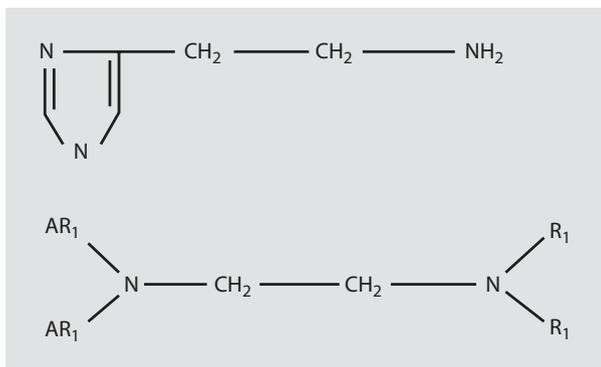
*First-generation antihistamines*, the oldest and nonselective, are used less often to treat allergies because they cause significant sedation and an anticholinergic side effect. They include:

- Diphenhydramine (Benadryl<sup>®</sup>, Dimedrol<sup>®</sup>)
- Clemastine (Tavegil<sup>®</sup>, Tavist<sup>®</sup>)
- Chloropyramine (Suprastin<sup>®</sup>)
- Cyproheptadine (Peritol<sup>®</sup>, Periactin<sup>®</sup>)

However, some of them are manufactured in ampoule form (see ■ Fig. 10.2) and may be used in urgent cases of allergy. First-generation antihistamines are usually prescribed for a short term, no longer than 10 days.

*Second-generation antihistamines*, newer and much more selective for peripheral H1 receptors, are currently used for allergy treatment. They do not cross the blood-brain

■ Fig. 10.1 Histamine (above) and Antihistamine



■ **Table 10.1** Histamine receptors

Receptor type	Expression	Effects
H1	Myocytes, endothelium, exocrine gland epithelium, neutrophils, eosinophils, monocytes, and macrophages	Itching Systemic vasodilatation Smooth muscle constriction (e.g., bronchospasm) Ileum contraction Mucus overproduction Stimulation of neutrophils and eosinophils, chemotaxis Increase in cGMP
H2	Exocrine gland epithelium, neutrophils, eosinophils, mast cells, and basophils	Stimulation of gastric HCl secretion Smooth muscle relaxation Inhibition of adaptive immune responses and cytokine production Sinus tachycardia Inhibition of neutrophils and eosinophils, chemotaxis Increase in cAMP
H3	Neurons, mast cells, and basophils	Decrease in acetylcholine, serotonin, and norepinephrine presynaptic autoreceptors
H4	Leukocytes, enterocytes, colonocytes, hepatocytes, splenocytes, thymocytes, and exocrine gland epithelium	Regulation of neutrophils and mast cells migration Involvement into eosinophil function

10



■ **Fig. 10.2** Suprastin and tavegil

## 10.2 • Antihistamines

barrier and act mainly in the periphery. However, analogous to the first-generation antihistamines, they are all “pro-medications” from which, in the course of biotransformation, active metabolites must be constituted. Second-generation antihistamines include:

- Azelastine (*Allergodil*<sup>®</sup>)
- Olopatadine (*Opatanol*<sup>®</sup>)
- Loratadine (*Claritin*<sup>®</sup>, *Alavert*<sup>®</sup>)
- Dimetindene (*Fenistil*<sup>®</sup>)
- Cetirizine (*Zyrtec*<sup>®</sup>) (see ■ Fig. 10.3)

*Third-generation antihistamines*, active metabolite derivatives of the second-generation drugs, are intended to have increased efficacy practically without side effects. They include:

- Fexofenadine (*Telfast*<sup>®</sup>, *Allegra*<sup>®</sup>) (see ■ Fig. 10.4)
- Desloratadine (*Aerius*<sup>®</sup>, *Clarinex*<sup>®</sup>) (see ■ Fig. 10.5)
- Levocetirizine (*Xyzal*<sup>®</sup>) (see ■ Fig. 10.6)

■ Fig. 10.3 Zyrtec



■ Fig. 10.4 Allegra 180 mg



■ Fig. 10.5 Aerius



■ Fig. 10.6 Xyzal



■ Table 10.2 Prescription of antihistamines depending on age

Age	Antihistamine
Since 1 month	Chloropyramine (Suprastin®) and dimetindene (Fenistil®)
Since 6 months	Cetirizine (Zyrtec®)
Since 12 months	Clemastine (Tavegil®, Tavist®) and desloratadine (Aerius®, Clarinex®)
Since 24 months	Levocetirizine (Xyzal®) and loratadine (Claritin®, Alavert®)
Since 6 years	Fexofenadine (Telfast®, Allegra®)

Second- and third-generation antihistamines are commonly prescribed for a longer term, up to 3 weeks.

Antihistamines are prescribed depending on age (see ■ Table 10.2).

■ Fig. 10.7 Fenistil Gel



■ Fig. 10.8 Opatanol eye drops, allergodil eye drops, and allergodil nasal spray

### 10.3 Prescription of Antihistamines in Pregnant Women

If a daily antihistamine is required during pregnancy, second- and third-generation drugs are preferred because they are less sedating and have a better side effect profile. Loratadine and cetirizine are preferred second-generation antihistamines for pregnant women because they are the safest and most effective. They are generally considered to be safe at recommended doses for the treatment of allergic rhinitis during pregnancy.

There are now antihistamines in the form of skin cream (see ■ Fig. 10.7), eye drops, and nasal spray (see ■ Fig. 10.8).

### 10.4 Membrane Stabilizers

*Membrane stabilizers*, ketotifen and cromones (sodium cromoglycate and nedocromil), act by blocking histamine release from mast cells and basophils at the early phase of atopic allergic inflammation. They can be a useful alternative to antihistamines in preventing allergic reactions. The indications to them are prophylaxis and treatment for allergic asthma, rhinitis, allergic rhinoconjunctivitis, and manifestations of food allergy,

as a part of the complex intervention. However, they take some weeks for the therapeutic effects to be seen.

## 10.5 Topical Corticosteroids

Corticosteroids are almost identical to the natural hormone, cortisol, which is produced by the body's adrenal glands. Corticosteroids, unlike antihistamines, can reduce reactions of both phases of atopic allergic inflammation, the early phase and late phase. They suppress the B-cell-mediated Th2-dependent response to allergens, inhibit the formation of new IgE molecules, downregulate both cellular forms of the allergic inflammation, with predominance of eosinophils and/or neutrophils, and prevent ongoing chronic allergic inflammation.

Nowadays, corticosteroids use directly in the particular area of the body where the allergy symptoms are being noticed. It targets the symptoms and minimizes any possible side effects from the treatment, allowing the physician to keep the dose of medication to a minimum. Topical corticosteroids use in the form of inhalers, nasal sprays, eye drops as well as gels, creams, and ointments.

*Avamys*<sup>®</sup> (*fluticasone furoate*) (see ■ Fig. 10.9) and *Nasonex*<sup>®</sup> (*mometasone furoate*) (see ■ Fig. 10.10) are used in the treatment of allergic rhinitis and conjunctivitis in patients over 2 years of age. However, nose and throat dryness or irritation, blood-tinged mucus/phlegm, and nosebleeds may occur as side effects during treatment.

Inhaled corticosteroids are an established therapy for bronchial asthma. Their success is based on their ability to improve control of asthma, avoid the use of oral corticosteroids, and probably to limit the risk of long-term disorder in lung function. There are

10

■ Fig. 10.9 Avamys nasal spray



■ Fig. 10.10 Nasonex nasal spray



■ Fig. 10.11 Pulmicort for inhalation



three basic types of devices that deliver inhaled medications including corticosteroids in asthma: (1) a metered-dose inhaler (MDI), which uses a chemical propellant to push the medication out of the inhaler; (2) a dry powder inhaler (DPI), which delivers medicine by a fast and strong inhalation; and (3) a nebulizer, which delivers fine liquid mists of medication through a tube or a “mask” under pressure.

For example, *Pulmicort*<sup>®</sup> (*budesonide*) (see ■ Fig. 10.11) is prescribed to children over 6 months of age and adult patients through a nebulizer (see ■ Fig. 10.13), whereas

*Flixotide*<sup>®</sup> (*fluticasone propionate*) (see ■ Fig. 10.12) is used in children over 12 months of age and adults through an inhaler. Inhaled corticosteroids are excellent medications for the treatment of asthma. However, inhaled corticosteroids have common side effects, which include sore mouth or throat, hoarseness, fungus infection in the mouth and lungs, coughing, a decrease in bone thickness, and fluid buildup in the eye.

Topical corticosteroids are currently the mainstay of treatment for atopic dermatitis. Skin topical forms include lotions, gels, creams, and ointments. In association with moisturization, the therapeutic effect may be good. Topical corticosteroids are categorized by action potency (see ■ Table 10.3). Steroid therapy may be discontinued when lesions disappear and are resumed when new rash elements occur.

■ Fig. 10.12 Flixotide inhaler



■ Fig. 10.13 Nebulizer



■ **Table 10.3** Topical corticosteroid skin form potency classes

Potency class	Generic name	Brand name	Concentration
Low	Hydrocortisone butyrate	Cortate® Unicort® Locoid®	0.1%, 0.5%, 1%, and 2.5%
Medium	Betamethasone valerate	Celestoderm® (see ■ Fig. 10.14) and Betnovate®	0.05% and 0.1%
	Mometasone furoate	Elocom® (see ■ Fig. 10.15)	0.1%
	Triamcinolone acetonide	Ftorocort® Aristocort D® Aristocort R® Vioderm-KC® Kenacomb®	0.025%, 0.05%, and 0.1%
High	Fluocinolone acetonide	Flucinar® Synalar® Synamol® Derma-smooth®	0.01%, 0.025%, and 0.01%
	Betamethasone dipropionate	Propaderm diprosone® Diprolene glycol® Akriderm®	0.025% and 0.05%
Highest	Triamcinolone acetonide	Aristocort C®	0.5%
	Clobetasol propionate	Dermovate®	0.05%

■ **Fig. 10.14** Celestoderm-V with Garamycin with garamycin



■ **Fig. 10.15** Elocom



The most common side effects of corticosteroids for skin use are observed mainly with long-term courses of treatment and include skin atrophy, striae, rosacea, perioral dermatitis, acne, and purpura. Side effects are unlikely to occur with short-term courses.

## 10.6 Calcineurin Inhibitors

Calcineurin inhibitors downregulate the IL2 gene transcription through the inactivation of transcription factor NFAT. It eventually leads to the reduced T-cell proliferation in the course of T-cell clonal expansion and correspondingly the involvement of new lymphocytes in inflammation. Topical calcineurin inhibitors, such as pimecrolimus (cream Elidel®) and tacrolimus (ointment Protopic®), are used in the short-term treatment for atopic dermatitis. Side effects of these medications may include the development of immunocompromised skin conditions.

## 10.7 Anti-leukotrienes

*Anti-leukotrienes* reduce the “late phase” of atopic allergic inflammation and mucus production and work in a similar way to corticosteroids but with fewer side effects. They include montelukast (Singular®) and zafirlukast (Accolate®).

# 10

## 10.8 Monoclonal Antibodies

Omalizumab (Xolair®) (see ■ Fig. 10.16), a recombinant humanized monoclonal anti-IgE antibody prescribed for persistent moderate-to-severe allergic asthma, has been

■ Fig. 10.16 Xolair



shown to improve asthma-related quality of life, decrease the number of courses of oral corticosteroids, and reduce the severity of recurrences. It is administered every 2–4 weeks subcutaneously, and improvement must be noticed after 4–6 months. To date, new medications on the basis of monoclonal antibodies directed to pro-inflammatory cytokines and their receptors are being developed to use for allergy treatment.

### ■ Quiz

Reading a question, please choose only one right answer.

#### ? Question 1

Antihistamines:

1. Compete with histamine for H1 receptors.
2. Split pro-inflammatory cytokines.
3. Inhibit IgE production.
4. Compete with histamine for H4 receptors.

#### ? Question 2

Diphenhydramine is related to:

1. First-generation of antihistamines.
2. Anti-leukotrienes.
3. Topical corticosteroids.
4. Second-generation of antihistamines.

#### ? Question 3

Loratadine refers to:

1. Third-generation of antihistamines.
2. Membrane stabilizers.
3. Second-generation of antihistamines.
4. First-generation of antihistamines.

#### ? Question 4

Membrane stabilizers develop the therapeutic effect:

1. Slowly.
2. Rapidly.
3. Immediately.
4. Unknown.

#### ? Question 5

Levocetirizine is related to:

1. First-generation of antihistamines.
2. Third-generation of antihistamines.
3. Membrane stabilizers.
4. Second-generation of antihistamines.

**? Question 6**

Some antihistamines may be even prescribed since the age of:

1. 12 years.
2. 2 weeks.
3. 1 month.
4. 12 months.

**? Question 7**

Antihistamines are the main type of medications for the therapy for bronchial asthma:

1. Yes.
2. Unknown.
3. No.
4. Probably.

**? Question 8**

Olopatadine is:

1. Antihistamine in the form of eye drops.
2. Anti-leukotriene in the form of capsules.
3. Topical corticosteroid in the inhaled form.
4. Topical corticosteroid in the form of nasal spray.

**10****? Question 9**

There are no topical corticosteroids in:

1. The cream form.
2. The ampoule form.
3. The form of nasal spray.
4. The inhaled form.

**? Question 10**

The topical corticosteroids exert anti-inflammatory effect:

1. At the systemic level.
2. In the liver.
3. On the particular area of the body.
4. In the genitourinary tract only.

**? Question 11**

Avamys® (fluticasone furoate) is used in the treatment for:

1. Bronchial asthma.
2. Atopic dermatitis.
3. Allergic rhinitis and conjunctivitis.
4. Urticaria.

**? Question 12**

Nasonex® (mometasone furoate) is used in the treatment for:

1. Allergic rhinitis and conjunctivitis.
2. Urticaria.
3. Atopic dermatitis.
4. Bronchial asthma.

**? Question 13**

Pulmicort® (budesonide) is prescribed through:

1. Metered-dose inhaler.
2. Dry powder inhaler.
3. Nebulizer.
4. Nasal spray.

**? Question 14**

Flixotide® (fluticasone propionate) is related to:

1. Antihistamines.
2. Topical corticosteroids.
3. Monoclonal antibodies.
4. Anti-leukotrienes.

**? Question 15**

Betamethasone dipropionate in the skin form exhibits:

1. High potency.
2. Low potency.
3. Medium potency.
4. Highest potency.

**? Question 16**

Xolair® is related to:

1. Topical corticosteroids.
2. Membrane stabilizers.
3. Monoclonal antibodies.
4. Antihistamines.

**Key Points**

Anti-allergy medications neutralize the mediators of allergic inflammation to result in the remission of allergic conditions. This approach linked to the anti-inflammatory therapy includes the use of antihistamines, membrane stabilizers, topical and systemic corticosteroids, and monoclonal antibodies directed to IgE, pro-inflammatory cytokines, and their receptors.

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# Allergen-Specific Immunotherapy (ASIT)

11.1 Introduction – 348

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**Electronic supplementary material** The online version of this chapter ([https://doi.org/10.1007/978-3-030-03323-1\\_11](https://doi.org/10.1007/978-3-030-03323-1_11)) contains supplementary material, which is available to authorized users.

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## Learning Objectives

*Knowledge.* Upon successful completion of the chapter, students should be able to:

1. Explain the mechanisms of action of allergen-specific immunotherapy (ASIT).
2. Be familiar with the allergen nomenclature.
3. Describe the allergic skin tests and other methods for allergy diagnosis.
4. Outline the main ASIT's protocols.
5. Describe indications, contraindications, and side effects of ASIT.

*Acquired Skills.* Upon successful completion of the chapter, students should demonstrate the following skills, including:

1. Interpret the knowledge related to atopic diseases and their treatment.
2. Critically evaluate the clinical literature about allergens and ASIT.
3. Discuss the scientific articles from the current research literature to criticize experimental and clinical data and formulation of new hypotheses in allergy.
4. Obtain a patient's history including history of present illness, past medical history, social, family, and occupational history, and review of systems.
5. Perform a patient's physical examination in a thorough manner.
6. Explain the rationale for choice of ASIT in a patient.
7. Attain a clear perception of the presented immunology definitions expressed orally and in written form.
8. Formulate the presented immunology terms.
9. Correctly answer quiz questions.

*Attitude and Professional Behaviors.* Students should be able to:

1. Have the readiness to be hardworking.
2. Behave professionally at all times.
3. Recognize the importance of studying and demonstrate a commitment.
4. Demonstrate the consideration of the patient's feelings, ethnic, religious, cultural, and social background, and be able to display empathy.

## 11.1 Introduction

The reader can find the up-to-date information on allergens and their nomenclature, subcutaneous and sublingual methods of allergen-specific therapy (ASIT), mechanisms of action of ASIT, clinical efficacy, etc. There is an explanation why the ASIT as a modality of treatment for atopic allergic conditions is more important and be predominant in the near future.

### Definitions

*Allergens* are antigens that induce the deviated forms of immune responses or sensitization described as types I–IV hypersensitivity.

*Allergen-specific immunotherapy (ASIT)* is the high-effective method of interventional immunology intended to the treatment for atopic diseases.

One of the two treatment modalities, allergen-specific immunotherapy (ASIT), is effective in reducing symptoms in selected patients with mild-to-moderate atopic allergic conditions. In addition, patients receiving ASIT for one condition may have a decreased risk of a so-called atopic march. For example, patients receiving ASIT for allergic rhinitis may be prevented from developing bronchial asthma.

Allergen-specific immunotherapy (ASIT) had come a long way since 1911 when L. Noon and J. Freeman showed the efficacy of ASIT in seasonal allergic rhinitis: “inoculations of allergen induce resistance.” This fact inspired a century of research in immunology. To date, multinational randomized placebo-controlled studies with children and adults of all atopic allergic conditions have demonstrated the clinically relevant efficacy and safety of ASIT. However, a new era of the ASIT is only just beginning.

*Mechanisms of action* of ASIT include the restoration of peripheral tolerance to causative allergens by reducing IgE production and modulation of the B-cell-mediated Th2-dependent response. Natural T-regulatory (nTreg) cells have been identified as key regulators of immunologic processes in the peripheral tolerance to allergens. These nTreg cells and induced Tr1 cells contribute to allergen-specific immune responses by suppression of the generation of effector B cells, downregulation of Th2 cells and Th22 cells, suppression of allergen-specific IgE, and induction of blocking IgG4 antibodies. They also inhibit the migration of mast cells, neutrophils, and eosinophils to the target tissues as well as the release of pro-inflammatory cytokines and other mediators of allergic inflammation.

In the *allergen nomenclature* based on (1) the Linnaean binomial nomenclature identifying genus and species and (2) modern advances in both sequencing and bioinformatics, an abbreviation of the scientific name of the allergen source, including genus (3 letters) and species (1 letter), follows first. For example, *Der p* means an allergen from house dust mite *Dermatophagoides pteronyssinus*. Next, one Arabic numeral (the number when this allergen was described among other allergens of this species) follows. After a period (.), the first two digits designate isoallergens, which are defined as allergens from a single species with similar molecular masses and biochemical functions and sequence identities >67%. The next two digits denote different variants of the isoallergen, which are defined as proteins with more than 90% sequence identity. The full allergen's name may look like *Der p 1.0101*, which we will be able to see on allergen vials for ASIT in the near future.

Historically, protein nitrogen units (PNU) were used when one PNU corresponded to 0.01 µg phosphotungstic acid-precipitable nitrogen, which stood for about 0.06 µg protein. Nowadays, various manufacturers use different allergen units, e.g., bioequivalent allergen unit (BAU), histamine equivalent prick test (HEP), etc. However, allergen standardization strategies should be uniform throughout the world to avoid confusion when estimating the potency of various allergen extracts. Taken in a simplistic form, all allergens may be divided into *major* and *minor* allergens. Major allergens can induce the strong IgE response, whereas minor ones trigger only the weak formation of IgE-synthesizing plasma cells. The major allergen must preferentially be chosen for any ASIT.

Allergenic molecules of the different sources (animals and plants) and routes of exposure, which are capable of inducing IgE response, constitute *allergome database* based on the literature published since the early 1960s. Low-molecular substances causing non-type I hypersensitivity, pseudo-allergic reactions, and/or intolerance are not any part of the allergome database. Allergen-associated molecular patterns (AAMPs)

are not included in the allergome database yet. You can find the information on any allergen at ► [www.allergen.org](http://www.allergen.org) if you know its biological source (i.e., the species' name).

A variety of allergen extracts currently use for both subcutaneous and sublingual immunotherapy.

*Indications* to ASIT include:

- Allergic rhinitis/rhinoconjunctivitis, allergic asthma, and atopic dermatitis when there is evidence of type I (atopic) hypersensitivity, during the period of remission
- Stinging insect (venom) allergy, during the period of remission

Additional indications to ASIT include:

- Impossibility to achieve control of allergic symptoms despite avoidance measures and pharmacotherapy
- Failure to escape from any contact with allergens
- Evidence of undesirable side effects with pharmacotherapy

*Contraindications* against ASIT are:

- Recurrence of atopic allergic conditions
- Uncontrolled or severe asthma
- Significant cardiovascular comorbid diseases (recent myocardial infarction, significant arrhythmia, and uncontrolled hypertension)
- Other significant comorbid diseases (malignancy, immunodeficiency, psychoses, and autoimmune diseases)

*Relative contraindications* against ASIT include using beta-blockers, pregnancy, the elderly, and children under 5 years of age (■ Figs. 11.1, 11.2, 11.3, 11.4, 11.5, and 11.6).

Evidence of the atopic origin of the allergy may be achieved by (1) *allergic skin tests* and/or (2) *allergen-specific IgE blood tests*.

■ Fig. 11.1 Staloral Sublingual/Oral Immunotherapy. Birch Allergen Extract



■ Fig. 11.2 Phostal Injectable.  
Birch Allergen Extract



■ Fig. 11.3 Alustal Injectable. House Dust Mites Allergen Extract

Because IgE antibodies have the cytophilic quality to be linked in the tissue, allergic skin tests are more appropriate, reliable, lowly invasive and allow, for most allergens, to obtain quick results. If the results of the prick or scratch tests are negative, they may be followed by intradermal tests, which give allergists more details about the causative allergens in a sensitized person. Skin tests are not performed for venom and drug allergies and are not recommended for diagnosing food allergies.

■ Fig. 11.4 Staloral Sublingual/Oral Immunotherapy. House Dust Mites Allergen Extract



11



■ Fig. 11.5 Alusta Injectable. 5 Grasses Mix Allergen Extract

Allergic skin tests (see ■ Fig. 11.7) are performed by a trained nurse in an allergist's office. Before the tests, medications such as antihistamines, membrane stabilizers, and anti-leukotrienes must be discontinued according to their half-life of

■ Fig. 11.6 Oralair Grass Pollen Sublingual Extract



■ Fig. 11.7 Skin test



elimination. When performing a prick or scratch test, a tiny drop of a possible allergen is pricked or scratched onto a certain area of the epidermis on the volar forearm, whereas for an intradermal test, a small amount of the potential allergen is injected by a thin needle inside a particular area of the epidermis. A prick/scratch test is assessed after 20 min taking into account the diameter of the wheal. An intradermal test is evaluated by diameter of the papule after 20 min (early phase) and 24 h (late phase). In both cases, histamine uses a positive control to assess the wheal reaction of the skin before testing.

For 100 years, conventional high-dose *subcutaneous* injection immunotherapy with allergen extracts continued to use as the standard gold therapy.

ASIT subcutaneous injections start with a minimal dose. The dose is gradually increased on a regular (daily or weekly) basis until an effective (maintenance) dose is reached. This commonly takes 1 to 6 months. This dose may vary individually. Once the maintenance dose is reached, injections are administered less often, usually monthly, although still on a regular basis. ASIT subcutaneous injections should always be administered in a medical facility by a trained nurse under an allergist's supervision. Also, a patient should remain at the medical facility for 60 min after the injection has been given.

A novel way of allergen administration is *sublingual*. The prescribed allergen is taken in the morning on an empty stomach. The drops or tablet should be kept under the tongue for at least 2 min and then swallowed. It is strictly prohibited to eat or drink anything for the next 15 min. If the daily dosage is missed, the patient must continue sublingual ASIT the following morning at the usual dosage.

It is recommended that ASIT is repeated for about 3–5 years, to reduce the likelihood that tolerance to the allergens will break down again.

**From a clinical viewpoint,** new strategies in the field of allergen-specific immunotherapy will progressively promote the efficacy and improve the safety of ASIT to make this method of therapy for allergies more broadly available to allergy patients, especially patients with more severe atopic asthma.

*Clinical efficacy* of subcutaneous and sublingual ASIT is demonstrated in well-designed clinical trials. It is not yet clear if sublingual immunotherapy is as effective as allergy injections. The efficacy includes:

- A disease-modifying effect
- Improvement of allergic symptoms even when they were resistant to conventional drug therapy
- Reduction of the risk for the future progression of mild atopic allergic conditions to moderate and severe forms
- Prevention of the onset of clinical atopy in atopic individuals with subclinical sensitization
- Reduction of using topical corticosteroids
- Prevention of severe systemic reactions to stinging insects, which may lead to a lethal outcome

*Side effects* during ASIT are usually local and mild and can commonly be eliminated by adjusting the allergen dosage. Anaphylaxis has occurred in rare cases and as a rule not “in professional hands.” Sublingual ASIT is safer than subcutaneous ASIT.

To prevent dangerous ASIT side effects, patients should:

- Stay at the medical facility for 60 min after the injection.
- Avoid exercising for at least 3 h afterward.
- Not use some heart and blood pressure medications without discussion with the prescribing allergist.
- Take an oral antihistamine before the injection.

**■ Quiz**

Reading a question, please choose only one right answer.

**? Question 1**

Allergen-specific immunotherapy (ASIT) may be used in treatment for:

1. Any allergic diseases.
2. Atopic allergic diseases only.
3. Disorders based on type IV hypersensitivity.
4. Disorders based on type III hypersensitivity.

**? Question 2**

ASIT is a therapeutic method, which leads to:

1. The restoration of natural tolerance to allergens.
2. The breakdown of natural tolerance to allergens.
3. The activation of innate immunity.
4. The initiation of T-cell-mediated adaptive responses.

**? Question 3**

ASIT is related to:

1. Evidence-based therapy.
2. Ancient methods of medicine.
3. Naturopathic medicine.
4. Chiropractic care.

**? Question 4**

ASIT is fulfilled by:

1. Nurse practitioner.
2. Lung doctor.
3. Allergist.
4. Dermatologist.

**? Question 5**

Immunosuppressive cytokines are:

1. IL4, IL5, and IL13.
2. IL6, TNF $\alpha$ , and TNF $\beta$ .
3. IFN $\gamma$  and IL2.
4. TGF $\beta$ , IL10, and IL35.

**? Question 6**

ASIT results in the induction of blocking IgG4 antibodies:

1. No.
2. Unknown.
3. Yes.
4. Probably.

**? Question 7**

ASIT also leads to:

1. Downregulation of Th2 cells.
2. Increase in production of IgE.
3. Upregulation of Th2 cells.
4. Increase in release of IL4.

**? Question 8**

Major allergens can induce:

1. The strong IgE response.
2. No IgE response.
3. Analogous to minor allergens.
4. The weak IgE response.

**? Question 9**

From an evolutionary viewpoint, “kings of allergens” are:

1. House dust mites.
2. Flour.
3. Insect venoms.
4. Pollens.

**? Question 10**

Allergen-associated molecular patterns (AAMPs) are responsible for:

1. Effective cross-linking of allergens by BCR/IgE.
2. Linking to a certain class of microbes.
3. Activation of inflammation and toxification.
4. Promotion of cancer growth.

**? Question 11**

Allergic skin tests are performed to:

1. Determine autoimmune disorders.
2. Confirm immunocompromised conditions.
3. Choose patients for ASIT.
4. Reveal type III hypersensitivity.

**? Question 12**

The “gold standard” of ASIT is:

1. High-dose subcutaneous injection immunotherapy.
2. Low-dose sublingual immunotherapy.
3. High-dose sublingual immunotherapy.
4. Intravenous immunotherapy.

**? Question 13**

This indication is not related to ASIT:

1. Atopic bronchial asthma.
2. Insect allergy.
3. Allergic contact dermatitis.
4. Atopic dermatitis.

**? Question 14**

Pregnancy is:

1. Relative contraindication against ASIT.
2. Obvious contraindication against ASIT.
3. Unknown.
4. Additional indication to ASIT.

**? Question 15**

Outdated allergen unit is:

1. Bioequivalent allergen unit (BAU).
2. Histamine equivalent prick test (HEP).
3. Protein nitrogen unit (PNU).
4. Biological unit (BU).

**? Question 16**

ASIT was developed by:

1. D. Bovet and E. Fourneau.
2. C. Richet and C. Pirquet.
3. A.F. Coca and R.A. Cooke.
4. L. Noon and J. Freeman.

**Key Points**

1. There are two treatment modalities, which may be used for patients with atopic allergic conditions, medication therapy and ASIT.
2. New strategies in the field of ASIT will progressively promote the efficacy and improve safety of ASIT to make this method more broadly available to allergy patients, especially patients with more severe atopic asthma. To the moment, there are not ASIT protocols for the entopic endotypes of atopy's disorders yet.

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# Supplementary Information

Answers to Quizzes – 360

# Answers to Quizzes

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## Chapter 1

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### Section 1.2

✓ 1. 3

✓ 2. 3

✓ 3. 1

✓ 4. 4

✓ 5. 2

✓ 6. 3

✓ 7. 1

✓ 8. 1

✓ 9. 4

✓ 10. 2

✓ 11. 4

✓ 12. 3

✓ 13. 1

✓ 14. 4

✓ 15. 2

✓ 16. 3

### Section 1.4

✓ 1. 2

✓ 2. 3

✓ 3. 1

✓ 4. 1

✓ 5. 3

✓ 6. 3

✓ 7. 1

✓ 8. 1

✓ 9. 4

✓ 10. 2

✓ 11. 2

✓ 12. 3

✓ 13. 1

✓ 14. 4

✓ 15. 2

✓ 16. 4

### Section 1.5.1

✓ 1. 3

✓ 2. 1

✓ 3. 4

✓ 4. 1

✓ 5. 2

✓ 6. 3

## Answers to Quizzes

✓ 7. 3

✓ 8. 1

✓ 9. 2

✓ 10. 2

✓ 11. 3

✓ 12. 1

✓ 13. 3

✓ 14. 2

✓ 15. 1

✓ 16. 4

**Section 1.5.2**

✓ 1. 3

✓ 2. 1

✓ 3. 4

✓ 4. 1

✓ 5. 2

✓ 6. 3

✓ 7. 3

✓ 8. 1

✓ 9. 2

✓ 10. 2

✓ 11. 3

✓ 12. 1

✓ 13. 3

✓ 14. 2

✓ 15. 1

✓ 16. 4

**Section 1.5.3**

✓ 1. 4

✓ 2. 1

✓ 3. 3

✓ 4. 1

✓ 5. 2

✓ 6. 3

✓ 7. 2

✓ 8. 1

✓ 9. 2

✓ 10. 2

✓ 11. 3

✓ 12. 2

✓ 13. 3

✓ 14. 1

✓ 15. 4

✓ 16. 3

**Section 1.5.4**

✓ 1. 2

✓ 2. 1

✓ 3. 3

✓ 4. 1

✓ 5. 2

✓ 6. 3

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**Section 1.5.5**

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**Section 1.6**

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## Answers to Quizzes

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**Section 1.7.2**

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**Section 1.7.3**

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**Section 1.7.6**

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**Section 1.7.7**

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**Section 1.7.9**

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**Section 1.7.10**

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**Chapter 2**

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**Section 2.2**

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**Section 2.3**

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### Section 2.4

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### Section 2.5

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## Chapter 3

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### Section 3.3

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## Answers to Quizzes

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**Section 3.4**

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**Section 3.5**

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### Section 3.7

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### Section 3.8

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## Chapter 4

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### Section 4.3

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## Answers to Quizzes

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**Section 4.4**

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**Section 4.5**

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**Section 4.6**

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#### Section 4.7

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**Section 4.9**

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**Section 4.10**

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**Chapter 5**

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**Section 5.10**

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## Chapter 6

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### Section 6.3

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### Section 6.4

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**Section 6.5**

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**Section 6.6**

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**Section 6.7**

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## Chapter 7

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## Chapter 8

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## Chapter 9

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## Chapter 10

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## Chapter 11

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## Ongoing Individual Life of the Immune System (Afterword)

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» Come in lo specchio sol, non  
altrimenti  
La doppia fiera dentro vi raggiava,  
Or con altri, or con altri reggimenti.  
Exactly like the sunlight in a mirror,  
The twofold bestia gleamed in her  
eyes,  
Now beaming with one nature, now  
the other.  
*Dante Alighieri. La Divina Commedia.  
Purgatorio. Canto XXXI, 121-123*

The immune system of each human being carries on as a specific individual process from immunonaissance until immunosenescence because it depends on a person's heredity, fetal life, environment after birth, nutrition, internal microbiota (microbiome), sex, age, seasonality, pregnancy (in women), and many other factors.

Studies have examined thousands of healthy volunteers and patients with various types of pathology to explain the differences in organs, cells, and molecules of the immune system at the individual level. Overall, genetic predisposition seems to have the most significant effect on the lifespan and strength of the immune system, with an influence that has been estimated from 25% to 75% among all factors. Quantitative specific loci mapping has identified the association with an increase in susceptibility to infections and immune-mediated diseases. Interestingly, variations in T-cell counts are more strongly dependent on genetic factors, whereas B-cell counts are more environmentally affected, especially in babies and children. From age 4 to 6 years, children do not yet have many mature B cells, which produce antibodies at the highest level required to defend against reactivated encapsulated bacteria.

The age-dependent involution of the thymus is the second most important factor contributing to the lifespan of the immune system. Some research groups even believe that the transplantation of a new thymus should be considered. Thymic involution leads to a progressively decreased output of naive T cells, which are responsible for the adaptive responses to and protection against newly invasive antigens. Consequently, the strength of the immune system diminishes with age, much like with an HIV infection, but not as fatally.

The immune system can be compared to purgatory, where souls are purified before going to heaven. The immune system can reliably and faithfully protect the body against invaders and tumors. However, in cases of progressing cancer, severe autoimmune diseases, and allergic disorders, the same immune system can betray the body. Unfortunately, a variety of problems of the body cannot be solved by the immune system, and the immune system is not only designed for protection. Some protein molecules that are important for the immune system are synthesized due to the transcription of both "good" and "bad" genes; consequently, the immune system may play both beneficial and harmful roles in the body. That is the dirty little secret of the immune system. As a tool of the hereditary program embedded in the individual genome, the immune system can trigger senescence processes at the appointed hour to lead the body to death. Such is the course of nature. However, while we are alive, we should look after our immune system to prolong its individual life and the lifespan of our bodies.