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► **To cite this version:**

Jacques-Olivier Pers, Panayiotis Vlachoyiannopoulos, Evangelia Zampeli, Haralampos Moutsopoulos. Basic Immunology. Immunology and Rheumatology in Questions, Springer International Publishing, pp.3-28, 2021, 10.1007/978-3-030-56670-8\_1 . hal-03105707

**HAL Id: hal-03105707**

**<https://hal.science/hal-03105707v1>**

Submitted on 30 May 2022

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# Basic Immunology

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## Abstract

Immune system provides the mechanisms for attacking foreign invaders, eliminating autologous toxic substances and offering self-tolerance.

A reductionist approach of the immune system components and their interactions as provided in this chapter will offer knowledge important to better understand the core mechanisms of autoimmune diseases and design therapies targeting their pathogenic mechanisms.

Immunity is divided into (a) innate (or natural), implemented by macrophages, dendritic cells, granulocytes (neutrophils, basophils, and eosinophils), natural killer cells, the complement system, and the acute-

phase proteins, and (b) adaptive, implemented by B and T cells. Immune cells express sensors on cytoplasmic or endosomal membranes or in the cytoplasm, called “pathogen-associated molecular pattern receptors” (PAMPs), also called pattern recognition receptors (PRRs), and damage-associated molecular pattern receptors (DAMPs) to sense foreign invaders or damaged tissues and provide defense against them. Natural immunity cells provide the first line of defense, usually successful in eliminating pathogens, but also they limber up the adaptive immune system to take action in case of any failure of defense. The sensors of B and T cells are their antigen receptors (membrane immunoglobulins and T-cell receptors, respectively) which are dissimilar to each other in terms of specificity; each receptor recognizes very specifically one antigen and especially a few peptide residues on it (epitope). However, by taking as a whole the pool of lymphocytes, their receptors offer a vast array of specificities exceeding  $10^{11}$  that is more than the genes of the human body. This implies that not particular genes but rather gene fragments are spontaneously rearranged to make an immunoglobulin or a T-cell receptor gene. One such rearrangement for each peptide chain of the receptor is allowed, so

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the cells will retain their antigenic specificity as long as they live. Immune cells communicate with each other and approach their targets, either by cell-to-cell contact using adhesion molecules or by soluble mediators known as cytokines or chemokines, respectively.

Mechanisms like central (taking place in bone marrow and thymus) and peripheral (taking place in the lymph nodes) tolerance ensure that the immune system will not attack self. Breaking of tolerance initiates autoimmune reactivity which may be subclinical but, under certain circumstances, may obtain a clinical phenotype.

**1. Which are the main Cluster of Differentiation (CD) molecules on the surface of immunocytes?**

CD molecules are cell surface markers that characterize different immune cell subtypes. In a simplistic way some CD molecules characterize the main immune cell subsets:

- CD4: T cells (helper/inducer), monocytes, macrophages, dendritic cells
- CD8: T cells (cytotoxic), NK cells
- CD14: monocytes
- CD15: granulocytes
- CD16: granulocytes and NK cells
- CD19: B cells (a component of the B cell receptor)
- CD20: B cells (type III transmembrane protein)
- CD22: B cells
- CD28: all T cells (co-stimulatory receptor)
- CD34: myeloblasts, lymphoblasts, endothelial cells
- CD38: plasma cells, B cells, T cells
- CD40: antigen-presenting cells (co-stimulatory protein)
- CD45: all leukocytes, all hemopoietic cells (except erythrocytes)
- CD57: T cells, NK cells, B cells, monocytes

**2. Which are the primary and secondary lymphoid organs?**

*Primary or central lymphoid organs* are those where lymphocytes are originally generated and educated to discriminate self from non-self; these include the bone marrow and thymus. *Secondary or peripheral lymphoid organs* are the lymph nodes, the spleen, and the lymphoid tissues of the gut, upper and lower respiratory tract, urogenital system, other mucosae, and arteries. Lymphocytes in these tissues are educated to react appropriately to foreign antigens in specific areas called germinal centers.

**3. Which are the components of the innate immune system?**

The innate immune system is constituted from:

- Physical barriers: Epithelial surfaces of skin, eye, oral, nasopharyngeal, respiratory, and gastrointestinal tracts, epithelial and phagocytic cell enzymes (i.e., lysozyme)
- Mucosal secretions: Sweat, tears, saliva, and gastric acid
- Antimicrobial peptides: For example, defensins, Pathogen-associated molecular pattern receptors (PAMPs)
- Damage-associated molecular pattern receptors (DAMPs)
- Cell receptors that sense microorganisms and signal a defensive response (e.g., Toll-like receptors)
- Cells: Polymorphonuclear leukocytes, macrophages, dendritic cells, natural killer cells, and mast cells
- Proteins: Acute-phase proteins, e.g., complement proteins and C-reactive protein
- The release of cytokines and chemokines

**4. Which are the main functions of the innate immune system?**

The innate immune system is evolutionary the oldest defense body mechanism. The immune

response that it generates is rapid, yet not highly specific, and does not generate an immunological memory.

Its main functions are:

- Removal of dead cells and foreign invaders via phagocytosis (macrophages, polymorphonuclear leukocytes, etc.)
- Recruitment of immune cells of the adaptive immune response
- Activation of the complement system cascade which promotes clearance of the foreign invaders, necrotic cells, as well as antigen-antibody complexes
- As long as the cells of the innate immune system phagocytose foreign invaders, they present antigens, acting as antigen-presenting cells, to the cells of the adaptive immunity and initiate specific immune responses

## **5. How do innate immune cells recognize foreign invaders?**

Cells of the innate immune system following exposure to bacterial or viral antigens ingest the foreign pathogen by phagocytosis. Structurally conserved microbe-associated molecules, known as pathogen-associated molecular patterns (PAMPs), are recognized by an array of specific sensors present in the plasma, plasma membranes, and host cytosol termed pattern recognition receptors (PRRs). The first classes of cellular PRRs to be identified were the transmembrane sensors called Toll-like receptors (TLRs). The nucleotide oligomerization domain (Nod)-like receptors (NLRs) and the retinoid acid-inducible gene-I (RIG-I)-like receptors (RLRs) are intracellular cytosolic sensors of PAMPs and danger-associated molecular patterns (DAMPs). RLRs are helicases that sense primarily viruses. NLRs can cooperate with TLRs and orchestrate the inflammatory and apoptotic response. TLRs are expressed on macrophages, dendritic cells, natural killer (NK) cells, B and T lymphocytes, as well as on non-immune cells like

epithelial and endothelial cells as well as on fibroblasts. Of the 10 human TLRs, six of them are located on the cell surface (TLR1, 2, 4, 5, 6, and 10) where they bind a diversity of molecule types, whereas TLR3, 7, 8, and 9 are in endosomes and sense nucleic acids. Each TLR recognizes different ligands:

- TLR1: Bacterial lipoproteins
- TLR2: Bacterial peptidoglycans and porins, virus envelope glycoproteins
- TLR3: Viral double-stranded RNA
- TLR4: Gram-negative bacteria (LPS) and host-derived HMGB1 and HSPs
- TLR5: Bacterial flagellin
- TLR6: Bacterial diacyl lipopeptides and lipoteichoic acid
- TLRs 7 and 8: Virus single-stranded RNA
- TLR9: Unmethylated CpG DNA of viruses and bacteria
- TLR10: Triacylated lipopeptides

## **6. What is the function of natural killer cells?**

Natural killer (NK) cells control the spread of several types of tumors and viral infections and thus limit the tissue damage. NK cells can also play a regulatory role by interacting with dendritic cells, macrophages, endothelial cells, and T cells. NK cells can thus limit or exacerbate immune responses. NK cells have the ability to distinguish the normal host cells through the killer cell immunoglobulin-like receptor (KIR) and CD94-NKG2A inhibitory receptors which recognize the Major histocompatibility complex (MHC) class I expressed on the surface of these normal cells. The binding of these receptors inhibits lysis and cytokine secretion by NK cells. In addition, NK cells have granules with perforins and granzymes that act on target cells inducing lysis or apoptosis and also express PRRs including TLR-2, -3, -4, -5, -7, and -8. Once activated, NK cells secrete interferon (IFN)- $\gamma$ , tumor necrosis factor (TNF)- $\alpha$  growth factors, interleukin (IL)-5, IL-10, IL-13, and chemokines.

## 7. Which are the subsets and functions of innate lymphoid cells?

Innate lymphoid cells (ILCs) were described as a branch of lymphoid lineage. They reside in tissues and are found in abundance in the intestinal lamina propria.

ILCs have been categorized into five subsets on the basis of their developmental pathways. In addition to NK cells and lymphoid tissue inducer cells, these are ILC1, ILC2, and ILC3. The characterization of the three ILC subsets was based on their cytokine production profile. ILC1 subset depends on the transcription factor T-bet, but not eomesodermin (EOMES) and, as well as NK cells, produces interferon (IFN)- $\gamma$  and is non-cytotoxic; ILC2 depends on GATA-binding protein 3 (GATA3) and produces IL-4, IL-5, and IL-13; and ILC3 depends on retinoic acid receptor-related orphan receptor- $\gamma$ t (ROR $\gamma$ t), and comprises subsets that can be distinguished on the basis of expression of the natural cytotoxicity receptors NKp46 (also known as NCR1) and NKp44 (also known as NCR2) and produces IL-17 and IL-22. Thus, their cytokine profile is similar to cytokine profiles of T helper (Th)1, Th2, and Th17/22 lymphocytes. Unlike T and B cells, ILCs do not express antigen-specific receptors derived from recombination-activating gene (RAG)-dependent gene rearrangements but are activated by cytokines produced by other innate immune cells as well as epithelial cells. ILCs exist in three differentiation stages as immature, naive, and primed. The immature ILCs express the CD5 molecule, the naive ILCs express the CD45RA molecule, while the primed ILCs express the CD45RO molecule. The ILC subsets have a huge plasticity with the ability to change phenotype and function according to the signals they encounter in the tissue they reside. The ILCs in addition to their traditional function as antimicrobial cell population play a significant role in maintaining tissue homeostasis and in regulating metabolic processes.

## 8. Which are the maturation, differentiation, and function of human mast cells?

Mast cells develop in the bone marrow but migrate as immature precursors that mature in

peripheral tissues, especially in the skin, the intestines, and the mucosae of the airways. This cell population is part of the first-line host defense against pathogens that enter the body across epithelial barriers. They are also involved in IgE-mediated allergic responses, since they carry a high-affinity Fc receptor for IgE immunoglobulin but remain inactive until at least two molecules of surface IgE will be cross-linked by binding the antigenic determinants of an allergen.

Mast cells are thought to serve at least three important functions in host defense. First, due to their location near body surfaces, they are able to recruit antigen-specific lymphocytes and non-specific effector cells, such as neutrophils, macrophages, basophils, and eosinophils, to sites where infectious agents are most likely to be encountered by the host. Second, by recruiting inflammatory cells, they cause intense inflammation which results in increasing the flow of lymph from sites of infection to the regional lymph nodes, where naive lymphocytes are first activated. Third, mast cell-derived leukotrienes (LTs) such as LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub> trigger muscular contraction and contribute to the physical elimination of pathogens from the lungs or the gut.

## 9. Which mediators are released from human mast cells?

- Reactive oxygen species (ROS)
- Preformed mediators stocked in their granules: serine proteases (chymase and tryptase), histamine, heparin, serotonin, and ATP
- Lysosomal enzymes:  $\beta$ -hexosaminidase,  $\beta$ -glucuronidase, and arylsulfatases
- Newly formed lipid mediators (eicosanoids): prostaglandin D<sub>2</sub>, leukotriene C<sub>4</sub>, thromboxane, and platelet-activating factor (PAF)
- Cytokines: Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and IL-1 $\beta$ , basic fibroblast growth factor (BFGF), stem cell factor (SCF), IL-4, and chemokines

## 10. Which are the complement proteins, their production and function?

The complement system includes over 30 proteins and/or protein fragments. These proteins or their fragments are in the serum, sero-

sal cavities, and cell membranes. They are generally synthesized by the liver and normally circulate as inactive precursors (pro-proteins). They constitute around 10% of the serum  $\gamma$ -globulins.

At sites of infection or inflammation, the complement system is sequentially activated through an enzyme-triggered cascade. The complement system, in order to perform its actions, is activated on large surfaces such as pathogen walls or large immune complexes, through three different ways: the *classical*, the *alternative*, and the *lectin pathways*. These pathways depend on different molecules for their initiation:

- The *classical* pathway involves complement components C1, C2, and C4 and is activated from antigen-antibody complexes binding to C1, which itself has three subcomponents C1q, C1r, and C1s. The pathway forms a C3 convertase (C4b2a), which splits C3 into two fragments: C3b (attaches to the surface of microbial pathogens and opsonizes them) and C3a (activates mast cells to release vasoactive substances such as histamine)
- The *alternative* complement pathway is activated by lipopolysaccharides on microbial cell surfaces in the absence of an antibody. It can also be triggered by foreign materials and damaged tissues. IgA complexes and the C3 nephritic factor (an autoantibody of C3 convertase) can also activate this pathway. It involves different factors (B, D, H, and I) that interact with each other and with C3b, to form a C3 convertase (C3bBb), which in turn activates more C3, leading to an amplification loop
- The *lectin* pathway is activated by the binding of mannose-binding lectin (MBL) to mannose residues on the pathogen surface. This further activates MBL-associated serine proteases, which activate C4 and C2, to form a C3 convertase (C4b2a)
- The final *lytic* pathway is initiated by the splitting of C5 into C5b. C6, C7, C8, and C9 unite with C5b forming the membrane attack complex (MAC), a multimolecular structure that inserts into the membrane creating a functional pore leading to cell lysis

## 11. When do antibody complexes lead to complement activation?

Large complexes are formed in great antibody excess and are rapidly removed from the circulation by the mononuclear phagocyte system and are therefore relatively harmless. The pathogenic complexes are of small or intermediate size (formed in slight antigen excess), which bind less avidly to phagocytic cells and therefore circulate longer. The mononuclear phagocyte system normally filters out the circulating immune complexes. Persistence of immune complexes in the circulation and increased tissue deposition occurs when macrophages are overloaded or have an intrinsic dysfunction. In addition, several other factors, such as charge of the immune complexes (anionic versus cationic), valence of the antigen, avidity of the antibody, affinity of the antigen to various tissue components, three-dimensional (lattice) structure of the complexes, and hemodynamic factors, influence the tissue deposition of complexes. A number of antibody-intrinsic factors are known to impact the ability of a given immunoglobulin to exhibit Antibody-Mediated Complement Activation (AMCA). The four subclasses of IgG (IgG1, IgG2, IgG3, and IgG4) display distinct complement activation profiles. In general, IgG1 and IgG3 are considered to be the most efficient activators, while IgG2 can be weakly activating, and IgG4 is often considered to be somewhat incapable of driving complement activation. Additionally, IgG Fc domains contain a conserved N-linked glycan in the vicinity of the C1q binding interface, which is required for efficient C1 activation.

## 12. Which are the key regulators of the complement system?

- C1 inhibitor: inactivates the proteases that associate with the recognition molecules of the *classical* and *lectin* pathways
- Complement receptor type 1 (CR1) (also referred to as CD35): is a membrane-bound complement inhibitor that can facilitate C3b/C4b degradation
- C4bBP: blocks the formation of C3 convertase in the *classical* pathway

- Membrane cofactor protein: blocks C3 convertase in both *classical* and *alternative* pathways
- Decay-accelerating factor: is anchored on cell membranes and promotes C3 convertase dissociation in both *classical* and *alternative* pathways
- Factor H: is the main regulator of the activity of the *alternative* complement pathway. Together with its splice variant factor H-like protein 1 (FHL-1), it inhibits complement activation at the level of the central C3b molecule, thus also blocks the amplification loop and the terminal pathway

**13. Which one of the activated complement components acts as vasodilator and which as chemoattractant?**

Proteins C3a, C4a, and C5a act as “anaphylatoxins.” They can trigger the degranulation of mast cells and basophils to release histamine resulting in increased vascular permeability and augmented inflammation. In addition, C5a is a

powerful chemoattractant for neutrophils and stimulates their degranulation. C5a and C3a also upregulate adhesion molecule expression on endothelial cells.

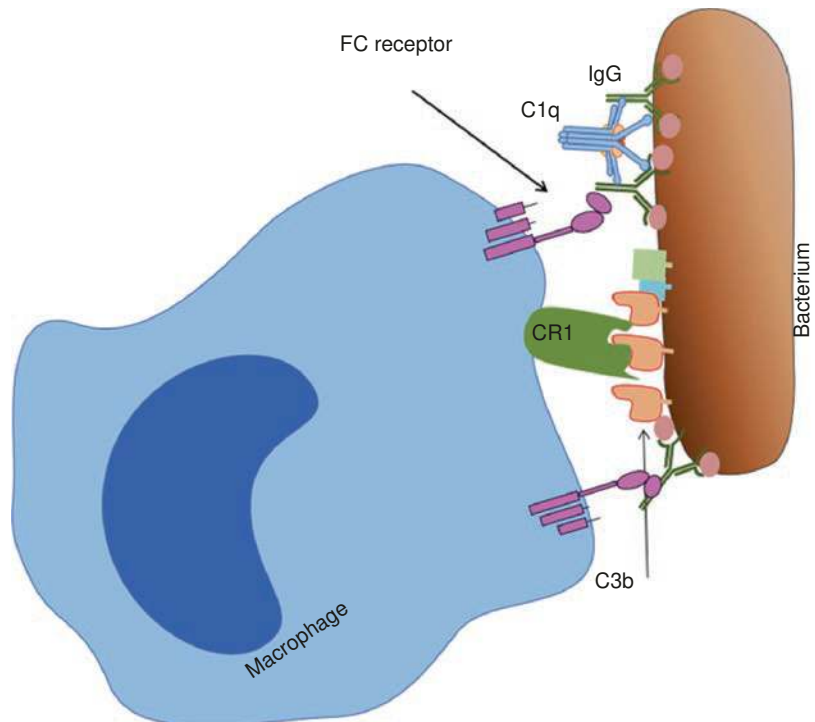
**14. What is opsonization?**

Opsonization is an immune process through which specific IgG antibodies or the C3b complement component (acting as an opsonin) bind to the surface of the foreign invader or the necrotic parts of host cells and the complex is being uptaken from the phagocytes through their Fc or C3b receptors (Fig. 1.1).

**15. Which cells can act as antigen-presenting cells (APCs)?**

Macrophages, dendritic cells, and B lymphocytes can act as professional APCs. The expression of MHC class II molecules along with co-stimulatory molecules and pattern recognition receptors (PRRs) is a defining feature of professional APCs. The non-professional APCs include

**Fig. 1.1** Opsonization: Bacteria opsonized by immunoglobulins and C3b complement component are phagocytosed when the Fc portion of antibody and C3b bind to their receptors on the surface of phagocytes (Figure created by Professor Panayiotis G. Vlachoyiannopoulos, MD)



all nucleated cell types in the body and typically express MHC class I molecules. However, it has been observed that antigen presentation to CD4<sup>+</sup> cells via MHC class II is not restricted to the classically professional APCs. Other leukocytes, including granulocytes, such as mast cells and neutrophils, can be induced to do so, as can endothelial and epithelial cells under certain circumstances, especially in autoimmunity.

### **16. What are the different subsets of monocytes?**

Monocytes are innate blood cells that maintain vascular homeostasis and are early responders to pathogens in acute infections. There are three well-characterized classes of monocytes: classical (CD14<sup>+</sup>CD16<sup>-</sup>), intermediate (CD14<sup>+</sup>CD16<sup>+</sup>), and non-classical (CD14<sup>-</sup>CD16<sup>+</sup>). Classical monocytes are critical for the initial inflammatory response, can differentiate into macrophages in tissues and can contribute to chronic disease. Intermediate monocytes are highly phagocytic cells that produce high levels of reactive oxygen species (ROS) and inflammatory mediators. Non-classical monocytes have been widely viewed as anti-inflammatory, as they maintain vascular homeostasis and constitute a first line of defense in recognition and clearance of pathogens.

### **17. What are neutrophil extracellular traps?**

Upon interaction with an invading microbe or cytokine discharge (IL-1 $\beta$ , TNF $\alpha$ , and IL-8), neutrophils release their chromatin material together with a wide range of granular enzymes to form net-like structures known as neutrophil extracellular traps (NETs). NETs cannot only trap the invading pathogen but also degrade them with NET-associated proteolytic enzymes. NETs accelerate the inflammatory processes by releasing a wide range of active molecules like danger-associated molecular patterns (DAMPs), histones, as well as active lytic enzymes in extracellular space, leading to further immune responses. Several of the molecules forming the NETs (e.g., myeloperoxidase, double-stranded

DNA, and histones) serve as autoantigens in systemic autoimmune diseases such as anti-neutrophil cytoplasmic antigen (ANCA)-positive vasculitis and systemic lupus erythematosus (SLE). Thus, several groups have proposed that in predisposed individuals aberrant NET formation plays a role in the generation of autoimmune responses. This hypothesis is supported by the observation that initiation or exacerbation of autoimmune responses often occurs following microbial infections.

### **18. How is tumor progression locus 2 (TPL2) kinase implicated in controlling inflammation?**

TPL2 is a mitogen-activated protein kinase (MAP3K). Recent evidence suggests that TPL2 positively regulates the isoforms p38 $\alpha$  and p38 $\delta$  of p38 MAPK on neutrophils.

p38 MAPK is a ubiquitous protein kinase that plays an important role in the inflammatory response. Stimulation of inflammatory cells, such as neutrophils, macrophages, and T lymphocytes, leads ultimately to a cascade of protein phosphorylation resulting in phosphorylation of p38. Phosphorylated p38 in turn causes production and secretion of pro-inflammatory cytokines such as interleukin 1 $\beta$  (IL-1 $\beta$ ) and tumor necrosis factor-alpha (TNF- $\alpha$ ).

In the absence of TPL2, macrophages have impaired production of inflammatory mediators such as IL-1 $\beta$ , TNF- $\alpha$ , IL-6, and IL-10. TPL2 has been shown to facilitate the migration of neutrophils into inflamed sites. In neutrophils, pharmacological inhibition of TPL2 selectively inhibits pro-inflammatory cytokines and chemokines via the MAPK and p38 pathways. In addition, TPL2 signaling is linked to the regulation of the adaptive immune system. In TPL2 knockout mice, it has been seen that TPL2 plays a role in Th1 differentiation and regulation of the inflammatory response in a T cell transfer model of colitis. TPL2 is also needed for MAPK signaling in B cells mediated by CD40 and/or BCR stimulation, which is important for immunoglobulin production.



**19. Which cell populations express the human leukocyte antigen (HLA) class I alloantigens and which the HLA class II?**

*HLA class I molecules:* are expressed on nearly all nucleated cells of the body. The antigens they present are peptide fragments endogenous to the cytoplasm of the cells expressing the MHC molecule, and they present them to CD8<sup>+</sup> T cells. The resultant T-cell response is cell-mediated killing or suppression of the MHC class I-presenting cell. Dendritic cells (DCs) and macrophages, can present exogenous antigens on MHC-I in a process called cross-presentation. This pathway plays a key role in antimicrobial and antitumor immunity, and also in immune tolerance.

*HLA class II molecules:* are expressed on professional APCs such as DCs, B cells, monocytes/macrophages, and any other activated APCs. In humans, HLA-DR, HLA-DQ, and HLA-DP molecules are the three classical and highly polymorphic MHC-II molecules. The antigens they present are peptide fragments present in lysosomal compartments as a result of phagocytosis or receptor-mediated endocytosis (e.g., bacterial material), and they present them to CD4<sup>+</sup> helper T cells. The resultant T-cell response is phagocytic and/or antibody response to eradicate the antigen presented.

**20. Which are the components of the adaptive immune system?**

Main components of the adaptive immune system are two subsets of leukocytes; the T and B lymphocytes. B cells and T cells are derived from the same multipotent hematopoietic stem cells and are morphologically indistinguishable from one another until after they are activated. These cell populations constitute the 20–40% of white blood cells (WBCs); their total mass is about the same with that of the brain or the liver. The majority of lymphocytes are in the lymphoid organs and in the tissues. B lymphocytes, through their products, the immunoglobulins, play a major role in humoral immune responses and can also function as APCs. T lymphocytes are the

major players in cell-mediated immune responses. The adaptive immune response is relatively slow yet highly specific, and additionally it creates immunological memory; in other words, after an initial response to a specific pathogen, the next encounter with the same antigen leads to an enhanced response to that antigen.

**21. Which are the steps of T-lymphocyte maturation and differentiation?**

T lymphocytes mature and differentiate in the thymus during an antigen-independent stage and later in the peripheral lymphoid organs during an antigen-dependent stage. Distinct stages of maturation and differentiation of T cells in the thymus are marked by the presence of surface molecules, namely, T-cell antigen receptor, CD3 protein complex (which acts as an adaptor to T-cell receptor), and the co-receptors, CD4, and CD8. More details on the developmental stages of T cells are the following:

- (a) Upon arrival from the bone marrow, T cells are negative for the T-cell antigen receptor and also negative for CD3, CD4, and CD8 surface molecules; these are called “double-negative T cells” or “double-negative thymocytes.” These T cells are still pluripotent
- (b) The double-negative thymocytes give rise to two different cell populations that can be distinguished from each other on the basis of the type of T-cell antigen receptor: the CD3<sup>+</sup> CD4<sup>-</sup>CD8<sup>-</sup>γδ T cells, which are a minority; these cells possess a T-cell receptor constituted of γ- and δ-chains (the repertoire of these chains is limited); however, the majority of thymocytes become CD3<sup>+</sup> CD4<sup>+</sup> CD8<sup>+</sup>αβ T cells (“double-positive” αβ T cells)
- (c) The CD3<sup>+</sup> CD4<sup>-</sup>CD8<sup>-</sup>γδ T cells move to the mucosal tissues. The double-positive αβ T cells remain in the thymus, enlarge, and continue to divide
- (d) The large double-positive αβ T cells undergo a stage of small, resting double-positive αβ T cells. These cells initially express low levels of αβ T-cell antigen receptors. Most of these

cells fail to recognize molecular complexes constituted of self-peptides bound to self-MHCs, and they die in the thymus. This process is called “positive selection”

- (e) The small double-positive  $\alpha\beta$  T cells whose receptors recognize molecular complexes constituted of self-peptides bound to self-MHCs (“positively selected” cells), lose either the CD4 or the CD8 co-receptors, and become “single-positive  $\alpha\beta$  T cells”
- (f) During the double-positive stage, but also after that, thymocytes which recognize with high-affinity molecular complexes of self-peptides bound to self-MHCs die, and the surviving cells are capable of responding to foreign antigens. This process is called “negative selection”
- (g) At this stage rearrangement of  $\alpha$ -chain locus begins and the level of expression, and the repertoire of surface  $\alpha\beta$  T-cell receptor increases. Thymocytes are now either CD3<sup>+</sup> CD4<sup>+</sup> CD8<sup>-</sup> $\alpha\beta$  T cells or CD3<sup>+</sup> CD8<sup>+</sup> CD4<sup>-</sup> $\alpha\beta$  T cells, which continue to mature inside the thymus for a while and then exit to periphery. These cells are called naive cells since they have not yet recognized an antigen
- (h) Upon recognition of foreign antigen in peripheral lymphoid organs, CD4<sup>+</sup>, as well as CD8<sup>+</sup>T cells, are differentiated to effector T cells; these are capable of leaving peripheral lymphoid organs and act within tissues

## 22. Which are the main subsets and functions of T lymphocytes?

T-lymphocyte subsets are:

- (a) CD3<sup>+</sup> CD4<sup>-</sup> CD8<sup>-</sup>  $\gamma\delta$  T cells home to mucosal tissues. They serve as an early-stage defense mechanism in mucosal surfaces. The actual diversity of  $\gamma\delta$  T-cell receptor is very limited. They are triggered by alarm signals such as heat shock proteins and several metabolites of pathogenic bacteria. Some subsets of CD3<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup>  $\gamma\delta$  T cells do not require antigen presentation through MHC, resembling thus cells of the innate immune

system. Yet, they have functions, particularly related to tissue homeostasis and wound healing

- (b) CD8<sup>+</sup> T cells. These cells are also called “cytotoxic T lymphocytes (CTLs).” They recognize cells infected by viruses (target cells). The target cells express MHC class I molecules in complex with viral antigens. CTLs kill them through a process called apoptosis. CD8<sup>+</sup> T cells in order to kill the target cell and reduce the burden of virus, release molecules such as perforins, granzymes, interferon- $\gamma$  (IFN- $\gamma$ ), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and express on their surface Fas ligand (Fas-L). Perforins are molecules which penetrate the membrane of infected cells; they are polymerized making holes in the membrane, while granzymes pass through these holes and enter the target cell; within the target cell, granzymes activate the caspase cascade, which eventually leads to programmed cell death known as “apoptosis”
- (c) T helper (Th) 1 cells. They recognize foreign antigens in complex with MHC class II molecules expressed on the surfaces of professional antigen-presenting cells (APCs). These cells contribute to killing of exclusively intracellular pathogens, such as bacteria entrapped into the phagosomes (*Mycobacterium tuberculosis* is a paradigm), as well as some viruses. The differentiation of naive T cells to Th1 phenotype is controlled by the cytokine interleukin (IL)-12. Cytokine signals combined with recognition of foreign antigens drive Th1 cells to secrete IFN- $\gamma$ . IFN- $\gamma$  will stimulate phagocytes to fuse the phagosomes with the lysosomes making the so-called phagolysosomes and thus destroy intracellular parasites
- (d) Th2 cells. They primarily stimulate immune responses against extracellular pathogens but also contribute to allergic reactions through stimulation of B cells. They provide help to certain B cells in order to produce IgE antibodies. They also secrete IL-4 and IL-13. Differentiation of Th2 cells largely depends on APCs which produce IL-4 that differentiates naive T cells to the Th2 phenotype

- (e) *Regulatory T cells* ( $T_{regs}$ ). These cells are generated when naive cells encounter antigen presented by cells that release transforming growth factor- $\beta$  (TGF- $\beta$ ). These cells produce also TGF- $\beta$  and IL-10 and downregulate immune responses.  $T_{regs}$  express cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and CD25 molecules on their surface as well as the intracellular protein FOXP3, which is a transcription factor
- (f) *Th17 cells*. These cells secrete IL-17, IL-22, and Granulocyte-macrophage colony-stimulating factor (GM-CSF). They participate in surveillance of fungi and extracellular bacteria as well as in the maintenance of barrier integrity. However, Th17 cells can become a pro-inflammatory cell subpopulation. Th17 cells are generated by CD4<sup>+</sup> naive T lymphocytes when they recognize an antigen expressed by a dendritic cell that produces TGF- $\beta$  and IL-6. In addition to IL-6 and TGF- $\beta$ , they are also stimulated by the cytokines IL-23 and IL-1 $\beta$ . Th17 cells contribute to autoinflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, psoriasis, and multiple sclerosis
- (g) *T follicular helper* ( $T_{fh}$ ) *cells*. These are CD4<sup>+</sup> T cells found within B-cell follicles of secondary lymphoid organs.  $T_{fh}$  cells are identified by their B-cell follicle-homing receptor CXCR5, which is constitutively expressed on their surface.  $T_{fh}$  cells express CD40L and secrete IL-21 and IL-4. Using the above molecules,  $T_{fh}$  cells trigger the formation and maintenance of germinal centers and contribute to the differentiation of antigen-stimulated B cells to plasma cells in order to enhance antibody production
- (h) *Th9 cells*. Th9 cells, a subset initially associated with the Th2 phenotype, are the primary source of IL-9. The production of IL-9 in Th9 cells is stimulated by TGF $\beta$  and IL-4 and inhibited by IFN- $\gamma$ . The production of IL-9 requires transcription factors that include STAT6 (signal transducer and activator of transcription 6), PU.1, IRF4 (interferon response factor 4), and GATA3. Th9 cells promote inflammation in a variety of

models but seem to be particularly capable of promoting allergic inflammation. Some of the effects of Th9 cells could be mediated through mast cells, but others are likely direct effects on tissue-resident cells

- (i) *Th22 cells*. Th22 cells are unique in that they express IL-22 but not IL-17 and IFN- $\gamma$ , and express the master transcription factor aryl hydrocarbon receptor. In addition to the production of IL-22, Th22 cells are characterized by surface expression of the chemokine receptors CCR4, CCR6, and CCR10 that are associated with cutaneous T cell homing. The major known functions of IL-22 are to regulate innate defense programs (e.g., antibacterial protein production), induce chemokine production, inhibit differentiation of some epithelial cells in the gastrointestinal and respiratory tracts, and stimulate tissue remodeling (Fig. 1.2)

### 23. What are MAIT cells?

Mucosal-associated invariant T (MAIT) cells are unconventional T cells that recognize microbial riboflavin-derivative antigens presented by the major histocompatibility complex (MHC) class I-like protein MR1. MAIT cells use a limited T cell antigen receptor (TCR) repertoire with public antigen specificities that are conserved across species. They can be activated by TCR-dependent and TCR-independent mechanisms and exhibit rapid, innate-like effector responses. In humans, peripheral MAIT cells are predominantly CD8<sup>+</sup> (~80%) or double negative (~20%) with a minor CD4-expressing population (~1%). MAIT cells are universally CD161<sup>hi</sup> (KLRB1, a C-type lectin-like receptor) and CD26<sup>hi</sup> (DPP4). The functioning of MAIT cells includes the potential for cytotoxicity, but they are especially known for their potent cytokine response. Stimulated MAIT cells rapidly release TNF, IFN- $\gamma$ , and IL-17, but most studies have reported that they do not produce Th2 cytokines.



*Follicular B cells* constitute the majority of B cells in the blood, lymph node, and spleen and are antibody-producing cells with a great array of specificities. They express surface immunoglobulins with a highly diverse array of specificities; they express also Fc receptors, MHC class II, as well as the surface molecules CD19 (a cell surface molecule in close junction with B-cell antigen receptor which decreases the threshold for antigen receptor-dependent stimulation) and CD23 (also known as FcεRII—a low-affinity receptor for IgE).

*Marginal zone B cells* constitute the minority of B cells in the blood, lymph nodes, and spleen and are also antibody-producing cells; they serve as the first line of defense against blood-borne pathogens, which reach the marginal zones through the circulation. They express surface immunoglobulin with limited receptor repertoire. These cells exhibit mainly antigen-independent stimulation, but they are also capable of undergoing antigen-dependent stimulation.

*B-1 cells* predominantly reside in the peritoneal and pleural cavity, produce natural antibodies, and exhibit T cell-independent activation. B-1 cells have been described in mice, and they overlap partially with CD5<sup>+</sup> B cells. B-1 cells have not been described in humans, but CD5<sup>+</sup> B cells exist. CD5 is a protein which reduces the severity of the activating signals derived from the B-cell receptors and makes B-1 cells to be activated by exogenous strong signals and not by signals derived from autologous tissues.

*Regulatory B cells* stop the expansion of pro-inflammatory lymphocytes through the secretion of IL-10, IL-35, and TGF-β while they promote the generation of T<sub>regs</sub>. The specificity of regulatory B cells and the exact way they differentiate are not known.

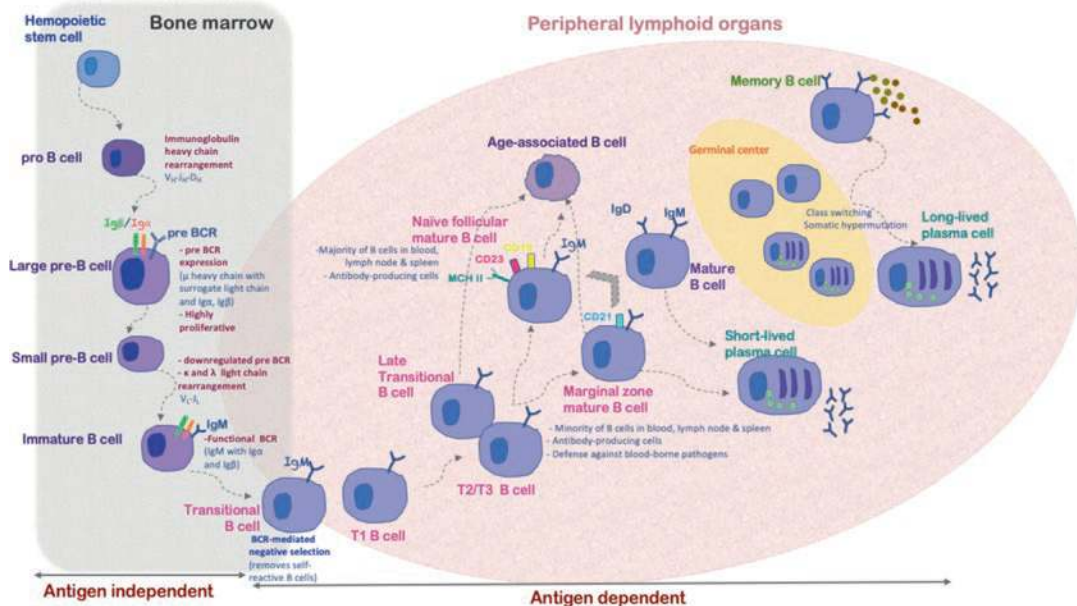
*Age-associated B cells* (ABCs) are a novel B lymphocyte subset whose numbers increase continuously with age and are refractory to B cell receptor (BCR) ligation but activated by TLR7 or TLR9 signals. ABCs can be generated from naive follicular B cells and possibly other pre-immune B cell subsets, such as marginal zone, B1, and transitional B cells. Their differentiation requires

TLR9 or TLR7 signals in the context of Th1 cytokines, particularly IFN-γ and IL-21. In contrast, Th2 cytokines such as IL-4 can impede ABC differentiation. ABCs contribute to immunological features of aging, including damped B cell genesis, altered immune responses to both primary and recall antigen challenges, and an increasing overall inflammatory climate. ABCs are elevated in autoimmune and autoinflammatory diseases (Fig. 1.3).

**26. Which are the maturation and differentiation steps of B lymphocytes? Which stage is antigen independent and which one is antigen dependent? What is antibody class switching and how is it achieved by B cells?**

The stages of maturation and differentiation of B lymphocytes (Fig. 1.3) are recognized by (a) distinct cell surface markers, (b) a specific pattern of immunoglobulin gene expression, and (c) cytoplasmic expression of transcription factors.

*Pro-B cells* express CD19 and CD10 surface molecules, while they retain the CD43 surface molecule expressed during the stem cell stage. CD43 is a transmembrane cell surface sialoglycoprotein on cells from hematologic lineage and contributes to ligand–receptor-induced T-cell activation. CD10 is a zinc-dependent metalloproteinase that cleaves and inactivates several hormones. During this stage, initiation of the expression of Rag-1 and Rag-2 (recombination-activating gene) proteins and the first recombination of immunoglobulin genes at the heavy-chain locus involving the D-J segment rearrangement take place. Rag-1 and Rag-2 proteins are important factors for the V-D-J rearrangement of immunoglobulin genes. The terminal deoxynucleotidyl transferase (TdT) that catalyzes the non-templated addition of junctional N nucleotides is also mainly expressed during the pro-B stage. Finally, the first C μ-exon of the C locus of the immunoglobulin heavy chain is rearranged with the VDJ segment, and a functional mRNA for the



**Fig. 1.3** Antigen-independent and antigen-dependent B cell developmental stages generating the distinct B lymphocyte subsets (Figure created by Evangelia Zampeli, MD)

heavy  $\mu$ -chain is produced and translated to immunoglobulin  $\mu$ -heavy chain. Only cells that achieve heavy-chain gene rearrangements survive. Pro-B cells do not respond to antigen.

*Pre-B cells* are the cells which have achieved a productive immunoglobulin  $\mu$ -gene rearrangement. These cells express immunoglobulin  $\mu$ -chain on their surface, but they have not rearranged gene segments of the immunoglobulin light chain. The immunoglobulin  $\mu$ -chain is covalently linked to surrogate light chains, which are invariant; that means they are the same in all pre-B cells. Pre-B cells synthesize also the  $Ig\alpha$  and  $Ig\beta$  chains of the B-cell receptor. Pre-B cells undergo tremendous proliferation and expansion of the B-cell lineage. The signal for proliferation is not known, but this is probably the process of linking of the surrogate light chains to the immunoglobulin  $\mu$ -chain. Activation of pre-B cell receptor leads to activation of Bruton's tyrosine kinase (BTK) which delivers signals for survival, proliferation, and maturation of pre-B cells, as well as of the B cells existing beyond this stage. At the pre-B-cell stage, three additional phenomena take place: (a) allelic exclusion, which means

any individual B cell expresses an immunoglobulin heavy-chain protein encoded only by one of the two inherited alleles, (b) stimulation of the  $\kappa$ -light-chain recombination, and (c) discontinuation of the surrogate light-chain transcription. Pre-B cell expresses also two surface markers, the CD43 protein (presented previously) as well as the CD220 protein—an insulin receptor with tyrosine protein kinase activity.

*Immature B cells*: these cells have already rearranged a  $\kappa$ -light-chain gene, and the  $\kappa$ -light chain associates with the  $\mu$ -protein; thus, immature B cells express fully assembled  $IgM$  immunoglobulin linked to the  $Ig\alpha$  and  $Ig\beta$  chains of the B-cell receptor, on their surface. Production of  $\kappa$ -protein prevents  $\lambda$ -rearrangement; therefore,  $\lambda$ -rearrangement occurs only in case  $\kappa$ -rearrangement was not productive. This phenomenon is called “light-chain isotype exclusion.” If both alleles of  $\kappa$ - and  $\lambda$ -chains fail to rearrange successfully, the B cell will die. The *RAG* gene expression is discontinued, and the cell prevents further rearrangement of the immunoglobulin genes. Immature B cells do not respond to antigens. In case their receptors bind

strongly to multivalent antigens in the bone marrow, the cells will die or sometimes they may undergo receptor editing—a process of modifying the sequence of light-chain V and J genes—so the cells are not further autoreactive and they can be rescued from programmed cell death. This phenomenon of eliminating strongly self-reactive B cells is called “negative selection.” Despite negative selection, there is no complete tolerance at the B-cell level; that means there are always autoreactive B cells in the periphery, but these cells need help from an antigen-specific T lymphocyte in order to respond to a certain antigen. Immature B cells survive the negative selection, leave the bone marrow, and reside in the spleen for a while to complete their maturation before reaching peripheral lymphoid organs.

*Mature B cells:* these cells divide into several subsets as described previously: follicular B cells, marginal zone B cells, B1/CD5<sup>+</sup> B cells, regulatory B cells, and age-associated B cells (ABCs).

Mature B cells that belong to the follicular B-cell subset produce high levels of IgM as well as IgD immunoglobulin using the same VDJ exon and the same  $\kappa$ - or  $\lambda$ -chain to express IgM and IgD surface receptors with the same antigenic specificity. These cells still have not encountered an antigen but have the armamentarium to respond to antigen and are called “mature-naive B cells.” Follicular B cells receive survival signals delivered by BAFF (called also BLyS) (B-cell activator or B-lymphocyte stimulator).

*Marginal zone B cells* are similar to B-1 cells described in mice; they express IgM and the CD21, which is the complement C3d receptor, identical to Epstein-Barr virus receptor. Unlike to what happens in mice, CD5<sup>+</sup> B cells in humans reside in both the spleen and the lymph nodes.

The stages of maturation of pro-B cells and pre-B cells are antigen independent. One could consider the negative selection observed at the immature B-cell stage as antigen dependent since these cells mature by encountering self-antigens in the bone marrow. However, immunologists agree that the word “antigen” in this context is used for exogenous antigens. Therefore, antigen dependent is only the maturation of naive B cells as soon as

they encounter antigens for the first time in the lymph nodes or other lymphoid tissues.

Antibody (or immunoglobulin) class switching is a biological mechanism which allows the antigen-activated B cell (which initially bears IgM surface immunoglobulin by which it recognizes the antigen), to produce immunoglobulins with the same immunoglobulin variable region, but this region is coupled to constant regions belonging to other isotypes, for instance, IgG, with the same antigenic specificity. This happens as soon as the B cell starts to proliferate after antigen recognition and presents (a) antigenic peptides coupled to MHC class II and (b) a molecule called CD40 on its surface. The above constituents are recognized by an antigen-specific T cell through its T-cell receptor and the CD40 ligand, respectively. T cell then releases a mixture of IL-4 and IL-5 which regulates immunoglobulin class switching by the B cell.

## **27. In which stages of B-lymphocyte maturation and differentiation is the CD20 molecule expressed?**

The CD20 molecule is expressed on the surface of late pro-B cells and continues to be expressed through all the stages of B-cell development (pre-B, immature B, mature B cells, as well as memory B cells) but not in plasmablasts or in plasma cells. Plasmablasts are immature plasma cells, and plasma cells are the effector B cells, which have been transformed into antibody-producing cells.

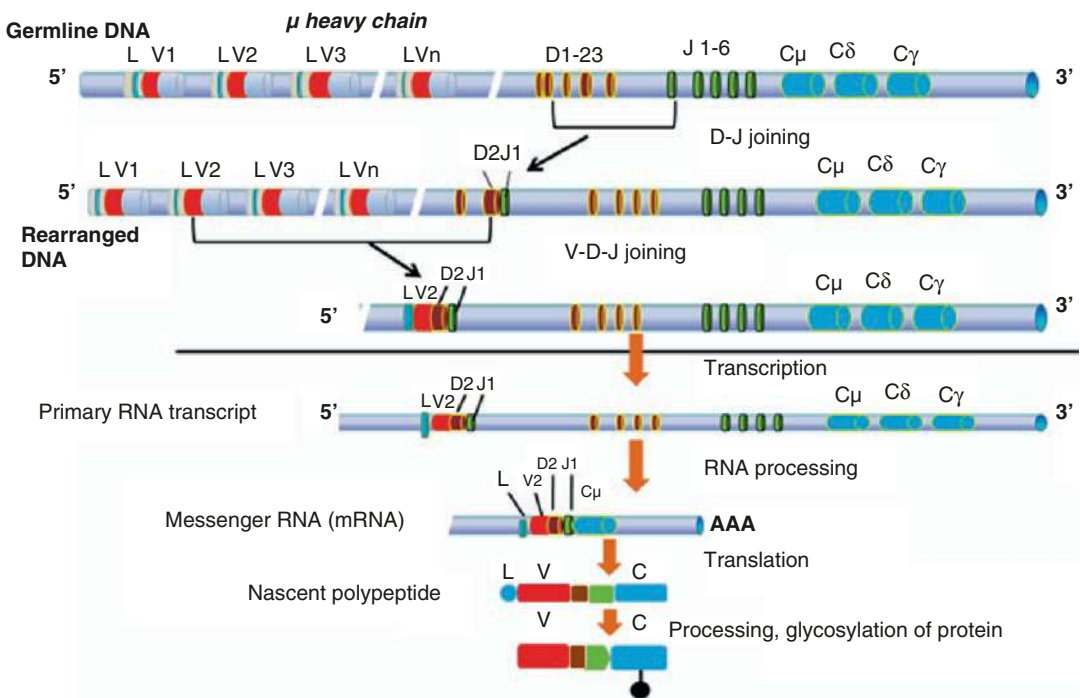
## **28. Which initial observations led to the postulation that antibodies are generated by “constant” and “variant” genes?**

Analysis of amino acid sequence of various immunoglobulins, nearly 50 years ago, revealed that many antibodies of the same isotype shared identical sequences at their C-terminal ends, but they varied considerably at their N-terminal ends, which, as we know today, correspond to the antigen-binding sites of antibodies. This finding led to the postulation that each antibody chain is encoded by at least two genes, one for the “constant” and one for the “variant” region and these genes are com-

bined at the DNA or RNA level constituting a “new” gene. DNA analysis in various tissues using markers of the “variant” (V) and “constant” (C) region revealed that in cells of any other origin except B lymphocytes, these regions were far away from each other within the genome and only in the B-cell DNA the V and C regions were closed, implying that during B-cell maturation and differentiation, a rearrangement of the immunoglobulin genes takes place and V and D regions are physically joint and translated to protein.

**29. Which is the organization of immunoglobulin genes and what are the mechanisms for gene rearrangement for the production of the entire immunoglobulin chains?** (Fig. 1.4)

We know today that three separate loci encode the immunoglobulin gene segments as follows: one locus for all the heavy-chain gene segments (located on chromosome 14), one for the  $\kappa$ -light-chain gene segments (located on chromosome 2), and one for  $\lambda$ -light-chain gene segments (located on chromosome 22). Gene segments lie in series at each locus from the 5' end to 3' end, encoding the variable (V) regions; at the 3' end of the series of the V gene segments, there are series of gene segments encoding the so-called diversity region (D); at the 3' end of them lie a series of gene segments encoding the so-called joining region (J); and at the 3' end of them lie a series of gene segments encoding the constant region (C). Light chains lack D regions, but their gene segments are organized



**Fig. 1.4** Organization of immunoglobulin genes and mechanisms for gene rearrangement: The organization of the  $\mu$ -chain gene segments is presented. Gene segments lie in series from the 5' end to 3' end, encoding the variable (V) regions; at the 3' end of the series of the V gene segments, there are series of gene segments encoding the so-called diversity region (D); at the 3' end of them lie series of gene segments encoding the so-called joining region (J); and at the 3' end of them lie series of gene segments encoding the constant region. The VDJ recombination in every individual B cell involves a random selection of one

V segment, one D segment (which is absent within the gene segments of the light chains), and one J gene segment to produce the VDJ exon. Therefore, a B cell may rearrange V1D2J2, another B cell may rearrange V2D3J5, etc. Every cell is capable of doing one gene rearrangement for the heavy and one for the light chain. Please note that after the VDJ gene segment rearrangement, the remaining rearrangements are performed at the primary RNA transcript level in order to produce a complete messenger RNA for the entire  $\mu$ -heavy chain (Figure created by Professor Panayiotis G. Vlachoyiannopoulos, MD)



in the same fashion, as that of the heavy chains. The heavy-chain locus contains nearly 45 V gene segments each of about 300 base pairs long, the  $\kappa$ -locus contains 30 V gene segments, and the  $\lambda$ -locus contains 45 V gene segments. The  $\kappa$ -light-chain locus has only one C gene segment, and the  $\lambda$ -light-chain locus has four C gene segments; the heavy-chain locus has nine C gene segments each encoding one immunoglobulin isotype (IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgE, and IgD). Each of the nine C gene segments is composed of five or six exons.

The VDJ recombination in every individual B cell involves a random selection of one V segment, one D segment (which is absent within the gene segments of the light chains), and one J gene segment to produce the VDJ exon. Therefore, a B cell may rearrange V1D2J2, another B cell may rearrange V2D3J5, etc. To complete the antigen-binding site, a VJ rearrangement among the light-chain gene segments should be also performed. Every cell is capable of doing one gene rearrangement for the heavy and one for the light chain; however, by taking together the whole B-cell population, a huge diversity of antigen receptors is generated. The C regions are located downstream of the rearranged VDJ exon (VJ exon for light chains) lying thousands of base pairs away. This part of the DNA is transcribed to RNA; RNA splicing contributes to deletion of the intervening RNA sequences and eventually brings together the VDJ exon (VJ exon for the light chains) with the C region exons; therefore, an mRNA is formed, which can be translated in the ribosomes to a complete immunoglobulin chain.

### 30. Which is the molecular mechanism through which the gene rearrangement for immunoglobulin production operates?

A thorough understanding of the mechanism of generation of antibody molecules needs to know the following: (a) the nature of recognition signals that drive VDJ recombination, (b) the enzymes involved in this process, and (c) the physical meaning of junctional diversity.

- (a) Certain DNA sequences called “recombination signal sequences” (RSSs) are located at the 3′ end of V gene segments, at the 5′ end of each J segment, and at both ends of each D segment. RSSs are highly conserved stretches of seven nucleotides (heptamers) located close to the coding sequence followed by a spacer of 12 or 23 nucleotides also highly conserved; this is followed by a highly conserved stretch of nine nucleotides rich in AT (nonamer). Double-stranded brakes are generated between the heptamer of RSS and the adjacent V, D, or J coding sequence. For the heavy chain, for instance, a DJ recombination occurs first by properly aligning the 3′ end of one D and the 5′ end of one J segment. This happens because two distinct heptamers come to the right position to be aligned with each other in a way that bridges the gap between the two gene segments. The intervening double-stranded DNA is removed in the form of a circular DNA. Same mechanism operates for recombination of the V with the DJ exon

The process of VDJ recombination can be divided into four distinct stages:

- *Synapsis*: this is the phenomenon of bringing together two selected coding segments and their adjacent RSSs by a chromosomal looping process; thus, the coding sequences are held appropriately for a recombination event
- *Cleavage* is the generation of double-stranded brakes at the RSS-coding sequence junctions by enzymatic cleavage. This is implemented by a complex of two enzymes in which one is activated by the other and both act together; these enzymes are the products of the recombination-activating genes 1 and 2 (*RAG-1* and *RAG-2*). These enzymes make nicks between coding regions and heptamers in each DNA strand, and the released 3′ OH of the coding end makes a covalent bridge to the phosphodiester bond of the other DNA strand, creating a hairpin
- *Hairpin opening and reprocessing*: the hairpins are opened up by an endonuclease called “Artemis.” Mutations of Artemis in mice are

causes of disrupted maturation of B and T cells leading to the generation of mice with severe combined immunodeficiency (SCID). After opening the hairpins, an enzyme called terminal deoxynucleotidyl transferase (TdT) adds bases to broken DNA ends and contributes substantially to a rather extent antibody diversity

- *Joining* is the process of ligation of the open ends of hairpins after TdT-induced processing. This process is called “nonhomologous end joining.” Several proteins contribute to this process, such as the proteins Ku70 and Ku80 which recruit to the open DNA ends the catalytic subunit of the enzyme DNA-dependent protein kinase (DNA-PK) which catalyzes a repair process. Mice deficient to DNA-PK are presented with the phenotype of SCID mice
- (b) The diversity of B-cell antigen receptors is attributed to two distinct processes: (a) combinatorial diversity which is attributed to the VDJ rearrangements and (b) junctional diversity which is operated by removal or addition of nucleotides at the junctions of the

V and D, D and J, and V and J segments, catalyzed by the enzyme TdT as previously described

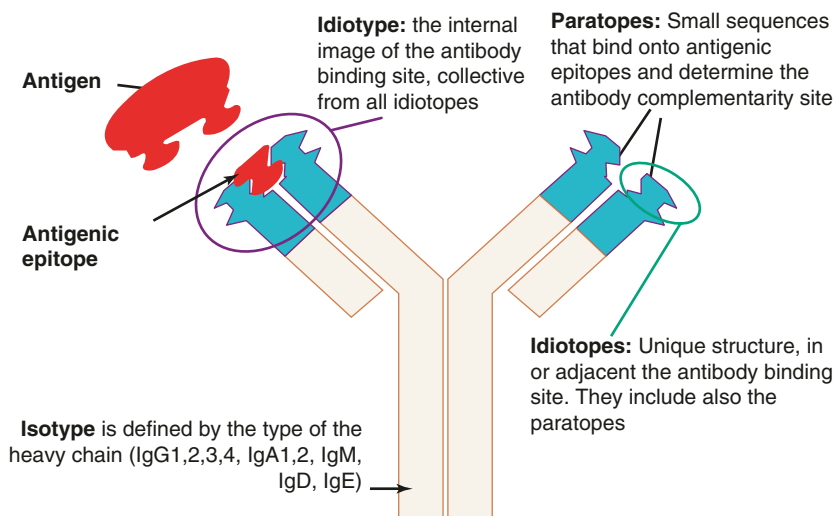
### 31. What are immunoglobulin idiotype, idiotope, paratope, and isotype? (Fig. 1.5)

*Idiotope* is the unique set of antigenic determinants (epitopes) of the variable portion of an antibody.

*Paratope* is a part of five to ten amino acids of an antibody’s variable region which recognizes and binds to an antigen. It contains parts of the antibody’s heavy and light chains.

*Idiotypes* are unique genetically controlled determinants which represent the antigenic specificity of the antibody. The idiotypic determinants are located into the immunoglobulin variable region of both immunoglobulin polypeptide chains.

*Isotype* defines the class of immunoglobulin, as it is determined by the type of its heavy chain. Immunoglobulin isotypes are IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgE, and IgD. Some authors use also the term “isotype” to differentiate the types of  $\kappa$ - and  $\lambda$ -light chains.



**Fig. 1.5** Immunoglobulin idiotype, idiotope, paratope, and isotype: Idiotopes are unique structures in the antibody-binding site or adjacent to this site. The collection of the entire number of idiotopes in a particular site of antibody-binding structure forms the idiotype; therefore,

idiotype represents the internal image of the antibody-binding site. Paratopes are small sequences that bind onto antigenic epitopes and determine the antibody complementarity site (Figure created by Professor Panayiotis G. Vlachoyiannopoulos, MD)

### 32. What is the function of the Fc portion of Immunoglobulin?

The Fc portion of immunoglobulins (Igs) contributes to the executive actions of antibodies; these actions are mainly dependent on the fact that many body cells possess receptors for the Ig Fc portion. The Fc functions can be classified as follows:

#### (a) Transportation of Igs:

- Polymeric IgA and IgM bind through their Fc portions to the polymeric immunoglobulin receptor (pIgR) secretory component, and they are transported across epithelial barriers
- The Fc portion of the maternal IgG binds to the neonatal Fc receptor in the placenta and facilitates IgG transport to the fetus

#### (b) Opsonization and phagocytosis of bacteria and immune complexes:

- Antibodies bind to bacterial cell wall polysaccharides and/or proteins and activate complement by interacting with the C1q, with the CH2 areas of the heavy Ig chains. Complement activation leads to the generation of C3b, which also binds to bacterial cell wall. This process is called opsonization. Opsonization is followed by bacterial phagocytosis which can be carried out by binding of the Fc portion of antibody and the C3b complement component to appropriate receptors on phagocytes
- Immune complexes enable the accumulation of many antibodies together; the CH2 areas of the heavy Ig chain of the antibodies upon binding to the C1q induce complement activation and generation of C3b; subsequently, the Fc portions of Igs along with the C3b complement component facilitate phagocytosis of immune complexes as previously described, and eventually immune complexes are destroyed

#### (c) The Fc portion of antibodies induces cellular signaling by binding to Fc receptors:

- Antibody-coated targets bind to Fc receptors on natural killer (NK) cells and activate these cells in order to destroy the target

- The Fc portion of IgE antibody binds with high affinity to IgE Fc receptors on mast cells and basophils and induces the release of enzymes and vasoactive amines from their granules
- Ig Fc portion through interaction with the appropriate Fc receptors on phagocytes, in addition to bacterial uptake, induces macrophage, neutrophil, and eosinophil signaling leading to activation of the respiratory burst and induction of foreign cell killing
- IgA and IgG which are specific for parasites are important for eosinophil binding via interaction with the eosinophil Fc receptors; then eosinophils then attack and kill parasites, by releasing toxic constituents from their granules. This process is known as “exocytosis”

### 33. What is the structure of secretory immunoglobulins?

Secreted polymeric immunoglobulins A and M (pIgA and pIgM, respectively) play vital roles in protecting the ~400 m<sup>2</sup> of human mucosa from invasion by pathogens. IgA and IgM contain 18-residue tailpiece extensions on their heavy chains that bestow polymer-forming capabilities. IgM can independently oligomerize to form hexamers, whereas IgA polymerization requires the 137-residue joining chain (JC). The predominant IgA oligomer is a dimer, though a smaller fraction of higher-order polymers up to pentamers have been described. Although less abundant than the dimer, higher-order polymers display better neutralizing capabilities, specifically for low-affinity antigens. The pIgA that is synthesized by mucosal plasma cells undergoes transcytosis, crossing the epithelium to reach the external secretions and perform its protective function. To initiate this process, pIgA binds the ectodomain of basolaterally expressed pIg receptor (pIgR), an interaction that requires the JC and is stabilized through a disulfide bond between the receptor and one Fc. Once bound, the receptor and pIgA undergo transcytosis through the epithelial cell to the mucosa. Upon transcytosis, proteolytic cleavage releases the secretory component (SC) of the receptor, which remains covalently attached to pIgA to form secretory

IgA (sIgA). In this mature form, sIgA performs its antimicrobial, neutralization, and protective functions.

#### **34. In which fraction of proteins do immunoglobulins belong?**

Several methods to perform serum or plasma protein fractionation exist. The most useful, easy, reliable, and clinically applicable in everyday medical practice is the serum protein electrophoresis, applied by Tiselius in 1937. Serum is applied to a trough (initiation point) on agarose gel or on a cellulose acetate membrane, and constant current is applied with a cathode to the site of the application of the serum. Serum proteins (which used to be called “globulins”) are separated by their size and electrical charge through electrical forces and electroosmotic forces. Proteins with negative charges such as albumin migrate to the anode, while proteins with positive charges remain close to the cathode. Using this technique, serum proteins can be fractionated in the following fractions (which are depicted as separate zones in electrophoresis), from the anode to the cathode: albumin,  $\alpha$ 1 globulins,  $\alpha$ 2 globulins,  $\beta$ 1 globulins,  $\beta$ 2 globulins, and  $\gamma$ -globulins.

Immunoglobulins migrate to the area of  $\gamma$ -globulins and in fact constitute a large part of the  $\gamma$ -globulin fraction, which appears as a smooth “blush,” or smear, close to the cathode. The majority of immunoglobulin molecules move to the cathode, but some of them move slightly toward the anode, so immunoglobulins occupy both sides of the initiation point. IgG moves toward the cathode and occupies the largest part of the  $\gamma$ -globulin fraction, together with IgM and IgD. However, IgA migrates to the anode and occupies the interzone between  $\beta$ - and  $\gamma$ -globulins as well as the  $\beta$  globulin zone.

#### **35. Based on what markers can lymphocyte populations be characterized as monoclonal?**

A monoclonal T- or B-cell population is defined as a collection of cells derived from a single precursor, and therefore, these cells share common characteristics to each other and also to

the precursor cell. In addition, monoclonal B or T cells derive from a pull of immature cells, and thus they may express surface molecules known as “clusters of differentiation” (CDs) which are unusual for healthy, mature cells. Light microscopy may also detect monoclonal B and T cells in case these cells possess morphological changes indicating an aggressive, non-regular phenotype. However, all these changes are suggestive of monoclonality, but they are nonconclusive. The following changes or markers—detected by various methods—indicate monoclonality:

##### *Immunopathological methods:*

*B cells:* a monoclonal B-cell population is defined by a monoclonal immunoglobulin on the surface of B cells. This monoclonal immunoglobulin should include only  $\kappa$ - or  $\lambda$ -light chains. Therefore, monoclonal antibodies to  $\kappa$ - or  $\lambda$ -light chains can differentiate a monoclonal B-cell population, defining B cells bearing only immunoglobulins which contain exclusively  $\kappa$ - or  $\lambda$ -chains.

*T cells:* there is not any immunopathological method to detect monoclonal T cells. T cell clonality is routinely evaluated with a PCR-based method using genomic DNA.

*Southern blot to detect B and T cell gene rearrangement:* the Southern blot is a sensitive and specific technique to detect monoclonal B- and T-cell populations by detecting precisely the same B- or T-cell receptor gene rearrangement in a collection of B or T cells, respectively. This technique can identify even small monoclonal cell populations compared to the total number of cells under study. Samples appropriate for analysis include fresh cell supernatants, fresh body fluids, or well-preserved frozen tissue sections.

#### **36. Which are the T and B lymphocyte receptors?**

T-cell antigen receptors are constituted of eight transmembrane polypeptide chains termed  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ , and  $\zeta$ . Each receptor possesses one  $\alpha$ -, one  $\beta$ -, one  $\gamma$ -, one  $\delta$ -chain, as well as two  $\epsilon$ - and two  $\zeta$ -chains. Chains  $\alpha$  and  $\beta$  are covalently linked to each other by a disulfide bridge between cysteine residues, close to the area of their emer-

sion from the cell surface. Each of  $\alpha$ - and  $\beta$ -chains contains one variable (V) and one constant (C) domain. Within the V domains, there are regions which differ significantly between individual T cells, and these are called “hypervariable (HV) regions” which come close to each other as the V regions of  $\alpha$ - and  $\beta$ -chains are folded; eventually, the HV regions form an area which is complementary to structures constituted of complexes of antigenic peptides with major histocompatibility complex (MHC) antigens; thus, the HV regions are called “complementarity-determining regions” (CDRs). The remaining transmembrane polypeptide chains are not involved in antigen recognition, but in intracellular signaling. They are joined to each other by charged residues as follows: one  $\epsilon$  is joined to  $\gamma$ , and one  $\epsilon$  is joined to  $\delta$ -chain, constituting the so-called CD3 complex, while  $\zeta$ -chains are mutually joined. The CD3 complexes, as well as the  $\zeta$ -chains, contain in their intracellular parts conserved sequences of four amino acids of which one is tyrosine; these motifs are called “immunoreceptor tyrosine-based activation motifs” (ITAMs) and facilitate signaling after interaction of the receptor with the MHC-antigenic peptide complex.

B-cell antigen receptor is constituted of membrane IgM or IgD immunoglobulins associated with two transmembrane invariant chains called Ig $\alpha$  and Ig $\beta$ , which are joined to each other by a disulfide bridge just after their emersion from the cell surface. Thus, the B-cell antigen receptor is the surface immunoglobulin, while Ig $\alpha$  and Ig $\beta$  contain ITAMs in their intracellular domains and facilitate signaling. After class switching, B cells as well as memory B cells express IgG, IgA, and IgE class surface immunoglobulins as antigen receptors.

### **37. How can B- and T-cell antigen receptors recognize millions of different antigens?**

In each individual B or T cell, a unique rearrangement of VDJ gene segments occurs and a unique antigen receptor emerges on their surface. Therefore, millions of different B or T cells exist, each expressing an antigen receptor of unique specificity. Thus, by taking B and T cells as a whole, they carry a huge diversity of antigenic receptors. As soon as a B or T cell encounters an antigen, the cell proliferates to make daughter

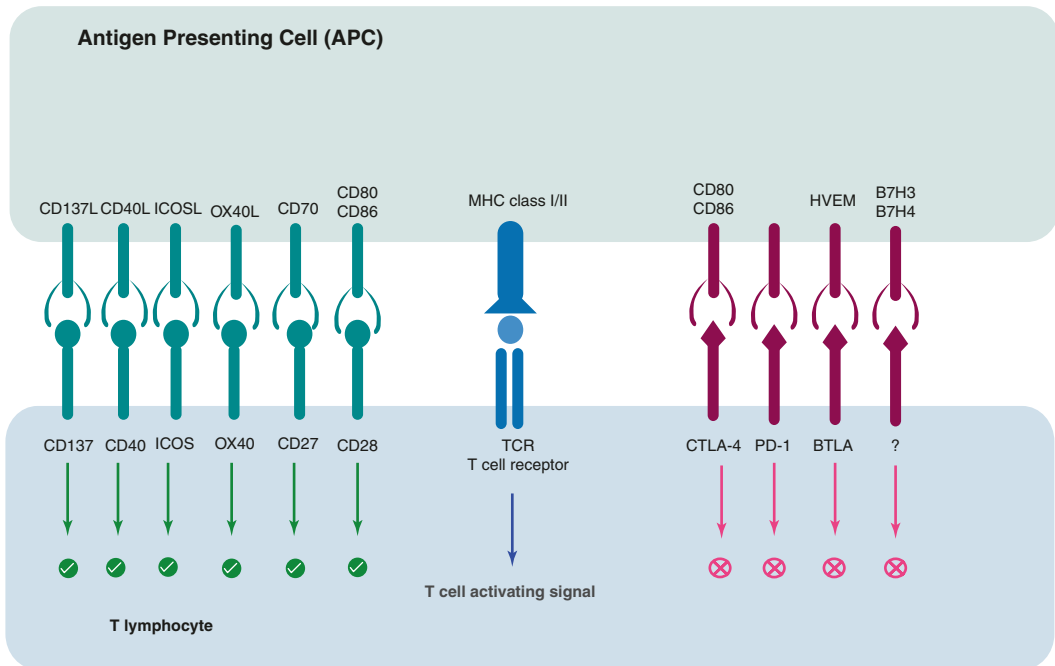
cells identical to the precursor cell. This phenomenon is called “clonal expansion.” The B cells differentiate to plasma cells and produce huge amounts of antibodies against the antigen they encountered, while T cells are transformed into “armed effectors T cells” (or simply “effector T cells”). Then T cells express their executive actions as helper, inflammatory, or cytotoxic cells in the injured tissues. The first encounter of antigen by T and/or B cells in order to successfully activate the cells for proliferation and differentiation should take place in the lymph nodes and other lymphoid tissues because these tissues provide the appropriate “environment” for T- and B-cell activation. Indeed, the lymphoid tissues contain soluble antigens drained by infected tissues, reaching the lymph nodes through the lymphatic vessels; in addition, antigen-presenting cells, such as dendritic cells coming from peripheral tissues and resident macrophages, present antigen “appropriately” to B or T cells; this means that antigen-presenting cells, in addition to antigen presentation, express co-stimulatory molecules and cytokines and provide T or B cells with additional signals for survival, proliferation, and differentiation.

### **38. Which are the main co-stimulatory molecules on the cell surface of lymphocytes and antigen-presenting cells?**

CD28 is expressed on T cells and interacts with CD80 (B7.1) and CD86 (B7.2) on antigen-presenting cells. The inducible T cell co-stimulator (ICOS, CD278) is expressed also on T cells and interacts with ICOS-Ligand.

Co-stimulation for B cells is provided alternatively by complement receptors (CR2). CR2 (CD21) on mature B cells formulates a complex with CD19 and CD81, which is often called B cell co-receptor complex because CR2 binds to opsonized antigens through attached C3d when the B-cell receptor binds the antigen.

CD40 is expressed on B cells and antigen-presenting cells which acts as a co-stimulatory molecule necessary for their activation. This is accomplished when it binds on its ligand CD154 (CD40L) on activated T helper cells. CD40 engagement on the surface of dendritic cells (DCs) promotes their cytokine production, the induction of co-stimulatory molecules on their surface, and facil-



**Fig. 1.6** Co-stimulatory and co-inhibitory molecules expressed by lymphocytes and antigen-presenting cells. Co-stimulatory molecules deliver positive signals to T cells (green) following their binding to ligands and receptors on APCs. Co-inhibitory molecules deliver negative signals (pink) to T cells upon interaction with their coun-

terparts on the APCs. The balance between co-stimulatory and co-inhibitory signals (red) alters the net stimulating effect of TCR signaling mediated through antigen presentation on MHC (blue) (Figure created by Evangelia Zampeli, MD)

terparts on the APCs. The balance between co-stimulatory and co-inhibitory signals (red) alters the net stimulating effect of TCR signaling mediated through antigen presentation on MHC (blue) (Figure created by Evangelia Zampeli, MD)

### 39. What are immune checkpoints?

Immune checkpoints (ICPs) are co-stimulatory and inhibitory signals transmitted by an assortment of receptors and their respective ligands (Fig. 1.6) in order to maintain immune homeostasis and self-tolerance. Under normal physiological conditions, ICPs are implicated in the initiation and in the determination of the intensity/duration of the immune response. They operate to limit collateral tissue damage during immune responses and therefore are critical for preventing autoimmune diseases.

Co-stimulatory checkpoint molecules (CD28, ICOS, CD27, CD40, OX40, and CD137) are nec-

essary to provide the “second signal,” after the interaction between the T-cell receptor (TCR) on the surface of T-cells and a major histocompatibility complex (MHC) molecule, in order to promote T cell activation. Absence of the co-stimulatory signal may lead to T cell anergy or apoptosis and decrease the strength of the immune response.

Co-inhibitory checkpoint molecules (CTLA-4, PD-1, BTLA, B7-H3, B7-H4, and VISTA) prevent T-cell activation. These checkpoints normally function to prevent exaggerated immune responses and subsequent autoimmune disease. Cancer cells can “hijack” immune checkpoints and escape immune destruction by acquiring abilities to inhibit immune checkpoint pathways and suppress anti-cancer T cell responses (Fig. 1.6).

### 40. Which are the mechanisms leading to the development of self-tolerance?

An outline of self-tolerance is as follows:

*Tolerance* is an antigen-dependent phenomenon. This implies that when immature B or T cells encounter self-antigens in the primary lymphoid organs, such as the bone marrow and thymus, they either (a) die through apoptosis or (b) change their receptor by a mechanism called receptor editing (a process which is applicable for B cells) or (c) become regulatory; that means the cell does not further react to self-antigens (a process which is applicable for CD4<sup>+</sup> T cells). The above processes constitute the so-called central tolerance.

In case self-reactive immature lymphocytes will be rescued from death in the thymus or bone marrow and migrate to the periphery, when they encounter self-antigens, they may (a) die through apoptosis, (b) become anergic throughout life, (c) be suppressed by regulatory T cells which have developed through central tolerance; or (d) finally, lymphocytes may ignore some antigens in the periphery, such as antigens of the testes or the eyes, which lie in immune-privileged sites. These processes constitute the so-called “peripheral tolerance.”

Mechanisms in detail, inducing tolerance, are as follows:

*Central T-cell tolerance:*

(a) Thymic medullary epithelial cells express many antigens exposed in the peripheral tissues under the influence of a protein which acts as a transcriptional regulator and promotes the expression of many antigens of peripheral tissues to thymus. This protein is called AIRE (autoimmune regulator); in the absence of AIRE, an autoimmune polyendocrinopathy syndrome is developed, because antigen-specific T cells for endocrine gland tissues escape central tolerance. AIRE does not explain the expression of so many antigens of the peripheral tissues in thymus, but it is a paradigm of how the system works. Central T-cell tolerance takes place in the thymus through the process of negative selection. This process affects both CD8<sup>+</sup> and CD4<sup>+</sup> T cells when their T-cell receptors bind with high affinity to “MHC/antigenic pep-

ptide” complexes, because in immature T cells, this high-affinity binding induces signals for apoptosis; on the contrary high-affinity T-cell receptor binding in mature cells induces signals for activation and proliferation

(b) Another option of central tolerance is the transformation of immature CD4<sup>+</sup> T cells in the thymus to regulatory T cells

*Central B-cell tolerance:* A range of mechanisms exist that collectively ensure that most self-reactive B cells are prevented from differentiating into plasma cells and thus from initiating pathogenic autoantibody production.

(a) Clonal deletion. B cells generated in the bone marrow and carrying BCRs that interact strongly with membrane-bound self-antigen expressed in this tissue can rapidly arrest maturation and die

(b) Receptor editing. Instead of directly triggering apoptosis, strongly self-reactive BCR specificities can be purged from the repertoire by the process of receptor editing. Strong cross-linking of BCRs on immature bone marrow B cells by self-antigen reactivates expression of their combinase-activating gene (RAG) proteins, thus triggering further VJ rearrangements within the immunoglobulin light chain genes. A new immunoglobulin light chain is expressed which offers a new (probably non-autoreactive specificity) to B-cell surface immunoglobulin (B-cell receptor)

(c) Clonal anergy. While both clonal deletion and receptor editing effectively remove self-reactive specificities from the primary B cell repertoire, many self-reactive B cells instead survive in the periphery in a functionally compromised state of anergy, characterized by desensitized BCR signaling and a suppressed ability to undergo plasma cell differentiation

(d) Clonal ignorance. Although not a tolerance mechanism per se, clonal ignorance describes the fate of self-reactive B cells that fail to encounter their self-antigen target, due to

either its scarcity or its sequestration inside cells or other inaccessible physiological structures

#### 41. How does peripheral T- and B-lymphocyte self-tolerance develop?

*Peripheral T cell tolerance:*

- (a) When autoreactive CD4<sup>+</sup> T cells encounter antigen in the periphery in the absence of co-stimulatory molecules, they become anergic; that is, they are not activated. There is some evidence that intracellular molecules important for T-cell receptor signaling are ubiquitinated, and the signal for T-cell activation is terminated. Another mechanism for autoreactive T-cell inactivation is the overexpression of CTLA4 surface molecule which belongs to the CD28 family, but when it binds to B7 molecules at the surface of antigen-presenting cells, it prevents B7:CD28 interaction and the T cell fails to be activated. Another molecule that belongs to the CD28 family and like CTLA4 inactivates autoreactive T cells is the PD-1. Ligands for PD-1 are expressed in antigen-presenting cells and other cells
- (b) Suppression of autoreactive T cells by T<sub>regs</sub><sup>+</sup>. The suppressive action of T<sub>regs</sub> is mediated by three mechanisms: (a) production of immunosuppressive cytokines IL-10 and TGF-β, (b) overexpression of CTLA4, and (c) consumption of IL-2, a phenomenon which results in deprivation of effector T cells which also need IL-2 for survival, proliferation, and activation
- (c) Issues regarding CD8<sup>+</sup> T-cell tolerance are not very well substantiated

*Peripheral B cell tolerance* is attributed to the following mechanisms:

- (a) Anergy and deletion which occurs when autoreactive B cells are repeatedly stimulated by autoantigen in lymphoid follicles. They need high levels of the growth factor BAFF/Blys; therefore they are unable to

compete normal naive B cells which can differentiate with much less amounts of BAFF/Blys, and eventually autoreactive B cells become anergic or die

- (b) Inhibition of autoreactive B cells by inhibitory molecules as depicted in mice but it is not substantiated in humans. Such molecules are CD22, Lyn, and SHP-1. The ligand for CD22 is not known

#### 42. What are the different cells interacting in a germinal center and their functions?

Different cell types collaborate to ensure positive selection of high-affinity germinal center (GC) B cells and differentiation into effector plasma cells and memory B cells.

- (a) Light zone and dark zone B cells. Light zone (LZ) and dark zone (DZ) domains within GC contain morphologically distinct populations of lymphocytes termed centrocytes (LZ, small, CD86<sup>hi</sup>CXCR4<sup>lo</sup>) and centroblasts (DZ, large, CD86<sup>lo</sup>CXCR4<sup>hi</sup>). Centroblasts and centrocytes are not separate B cell lineages but represent alternating phenotypes adopted by GC B cells as they move through the cyclic processes that underpin positive selection in the GC
- (b) Follicular dendritic cells. FDCs are specialized, nonhematopoietic cells that derive from perivascular precursors, form the nucleation site for nascent GCs, and are essential for both initiation and maintenance of GCs. As B cell proliferation continues and the GC matures to form LZ and DZ compartments, FDCs localize exclusively to the LZ. Their primary role is to act as a depot for intact antigen, which they maintain for extended periods (weeks to months) on their plasma membrane in the form of immune complexes or opsonized macromolecules
- (c) T follicular helper cells. As with GC B cells, T follicular helper (T<sub>fh</sub>) cells derived from the original antigen-specific lymphocyte clones responsible for initiating the T-dependent B cell responses that ultimately result in GC formation. Like FDCs, T<sub>fh</sub> cells



are found almost exclusively within the LZ of the GC, where they act as the source of T cell help that sustains the ongoing proliferation and selection of GC B cells. In addition to  $T_{\text{h}}$  cells, a related population of  $\text{Foxp3}^+$  T cells designated T follicular regulatory ( $T_{\text{fr}}$ ) cells has also been identified within GCs and seems to suppress GC responses at least under some circumstances

- (d) Tingible body macrophages. Tingible body macrophages (TBM $\phi$ s) are phagocytic cells unique to the GC that, although relatively rare, have a prominent phenotype characterized by internal tingible bodies corresponding to lymphocytes in various states of degradation. TBM $\phi$ s are typically found in close proximity to the border between the LZ and DZ and act as the GC's garbage disposal system, removing there mains of the large numbers of B cells that undergo apoptosis in the GC

#### 43. What are tertiary lymphoid structures?

Tertiary lymphoid structures (TLS) or ectopic lymphoid structures often occur at sites of autoimmune inflammation, organ transplantation, and cancer. Unlike secondary lymphoid organs, TLS lack a capsule and afferent lymphatic vessels and they are transient structures, which resolve after antigen clearance. TLS are characterized by B- and T-cell segregation, ectopic expression of lymphoid chemokines CXCL13, CCL19, and CCL21 that regulate T and B lymphocyte compartmentalization, high endothelial venules, and often by the presence of  $\text{CD21}^+$  follicular dendritic cell (FDC) network which sustains the local humoral B-cell response. TLS can display features of active germinal centers (GCs), including the expression of the enzyme activation-induced cytidine deaminase (AID) which regulates immunoglobulin gene affinity maturation via the process of somatic hypermutation, and they support a local antigen-driven B-cell proliferation and differentiation into antibody-producing plasma cells. TLS support the local proliferation and differentiation of autoreactive B cells in a disease-specific manner suggesting that the maintenance of TLS is also sustained, at least

in part, by an autoantigen-dependent process with the differentiation of autoreactive plasma cells toward disease-specific autoantigens. Recently, TLS have been associated with a favorable response to immunotherapy.

#### 44. Which are some examples of pro-inflammatory cytokines and the cells that produce them?

*Tumor necrosis factor- $\alpha$* : produced by macrophages, T cells, mast cells, and NK cells. Its main functions are increase of adhesion molecules, stimulation of the production of interleukin (IL)-1 and IL-6, activation of neutrophils, and initiation of acute inflammatory response.

*IL-1*: produced by macrophages, dendritic cells, neutrophils, and endothelial cells. Its main functions are increase of adhesion molecules and stimulation of hepatocytes to produce acute-phase reactants.

*IL-12*: produced by dendritic cells and macrophages. Its main functions are to increase IFN- $\gamma$  production, transform  $\text{CD4}^+$  T cells to Th1 phenotype, and increase cytotoxic activity of NK cells and  $\text{CD8}^+$  T cells.

*IL-6*: produced by macrophages, endothelial cells, and fibroblasts. Its main functions are maturation of B cells and antibody production, differentiation of neural cells, and stimulation of hepatocytes to produce acute-phase reactants.

#### 45. What is the cytokine release syndrome?

Cytokine release syndrome (CRS) or cytokine storm syndrome is a systemic inflammatory response, which can be caused by infections, some drugs, and other factors, characterized by a sharp increase in the level of a large number of pro-inflammatory cytokines. CRS is more common in immune system-related diseases or immune-related therapy, such as Chimeric antigen receptor (CAR)-T cell therapy, organ transplantation sepsis, and virus infection (i.e., SARS-CoV2). CRS can be induced by direct target cell lysis with consecutive release of cytokines like interferon-gamma (IFN- $\gamma$ ) or tumor necrosis factor-alpha (TNF- $\alpha$ ) or by activation of T cells due to therapeutic stimuli with subse-

quent cytokine release. These cytokines trigger a chain reaction due to the activation of innate immune cells like macrophages and endothelial cells with further cytokine release. IL-6 contributes to many of the key symptoms of CRS.

#### 46. Which are some examples for the function of Th1, Th2, and Th17 cells and cytokines?

*Th1 cytokines:* IFN- $\gamma$ , IL-2, and TNF. They are produced by CD4<sup>+</sup> Th1 cells. Their main functions are cell-mediated immunity and phagocyte-dependent inflammation.

*Th2 cytokines:* IL-4, IL-5, IL-6, IL-10, and IL-13. They are produced by CD4<sup>+</sup> Th2 cells and innate lymphoid cells-2 (ILC-2). Their main functions are to evoke strong antibody responses (including IgE) and eosinophil accumulation (phagocyte-independent defense) and inhibition of Th1-mediated macrophage activation.

*Th17 cytokines:* IL-17, IL-22, and IL-21. They are produced by Th17 cells. Their main functions are recruitment of neutrophils, induction of antimicrobial peptides in epithelial tissues, and stimulation of inflammation.

#### 47. What is the interferon (IFN) signature?

Multiple type I interferons (consist of 13 IFN- $\alpha$  subtypes, IFN- $\beta$ , IFN- $\omega$ , IFN- $\kappa$ , and IFN- $\epsilon$ ), interferon- $\gamma$  (IFN- $\gamma$ ) and type III IFNs (IFN- $\lambda$ 1 (IL-29), IFN- $\lambda$ 2 (IL-28A), IFN- $\lambda$ 3 (IL-28B), and IFN- $\lambda$ 4) are expressed under physiological conditions and are increased by stress and infections, and in autoinflammatory and autoimmune diseases. Interferons activate the Jak-STAT signaling pathway and induce overlapping patterns of expression, called “interferon signatures,” of canonical interferon-stimulated genes (ISGs) encoding molecules important for antiviral responses, antigen presentation, autoimmunity, and inflammation. Although the three IFN types signal via distinct receptors, the genes or signatures controlled by these IFNs overlap substantially. Although the potential pathogenic roles of IFN signatures in autoimmune diseases has been highlighted, both type I and II IFNs induce negative feed-

back and inhibitory pathways and have been implicated in suppressive effects, such as immune cell exhaustion. Thus, IFNs are likely to mediate both pathogenic and protective mechanisms in autoimmune diseases.

#### 48. Which cells produce chemokines and what is their function?

Chemokines are produced by leukocytes and by several types of tissue cells such as endothelial cells, epithelial cells, tissue macrophages, fibroblasts, and other cells of the stroma, under the stimulation of cytokines like TNF and IL-1 $\beta$  or under the recognition of pathogen-associated molecular patterns.

Chemokines are divided into two major subfamilies defined as CXC (when two cysteine residues are separated by every other amino acid), which are called  $\alpha$  chemokines, and the CC (when two cysteine residues are adjacent), which are called  $\beta$  chemokines. A subgroup of CXC chemokines (for instance, CXCL8, also termed IL-8) with the sequence “glutamic acid-leucine-arginine” (ELR) motif just before the CXC motif support neutrophil migration to infected or damaged tissues. The remaining CC (for instance, CCL2, also termed MCP-1) and CXC (for instance, CXCL10, also termed IP-10) chemokines support the migration of monocytes, lymphocytes, and other immune cells.

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