Overview of Immune Responses

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1 General Aspects of the Immune System

The immune system is classically described as the network responsible for the 'protection' of our body against foreign substances or microorganisms, infectious or non-infectious. The immune responses are usually initiated by products or constituents of bacteria, viruses, fungi, parasites, or any other substance recognized as non-self, although self-components are also able to trigger immune reactions under specific situations. Even though the 'defense' is in fact a major function, it is well known nowadays that the physiological role of the immune system is much broader and includes tissue repairment, tolerance and antitumoral immunity. All these activities depend on a process called **recognition**, that results from the interaction between soluble and cell-associated receptors—immune sensors of the microenvironment—and their respective ligands. When a receptor-ligand interaction occurs, a series of molecular signals are initiated followed by the activation of biochemical cascades that culminate with the production of effector molecules and mediators that regulate the cellular activity. The net result of these multiples interactions produces **immunity** or **tolerance** states. However, when these interactions are deregulated, they may also cause **hypersensitivity** or **autoimmune** reactions.

Didactically speaking, the most common division used to describe the immune system takes into consideration the time required to the response against an aggressor agent to be initiated. Thus, the **innate (natural, native) immunity** is responsible for the responses that occur immediately or soon after the first contact with any given microorganism or its products [24]. The cells and molecules of the innate immunity are ready to perform its functions at the time of recognition of the attacking agent, or immediately following this contact. On the other hand, the **adaptive (acquired)**

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immunity takes a few days to be fully developed and its effector mechanisms are mediated by cells called lymphocytes and their products [12]. In some cases, both lymphocytes and the molecules they produce are able to act directly on an extracellular microorganism or a cell infected with an intracellular microorganism. In other situations, lymphocyte-derived products amplify the effector mechanisms of the already existing innate immunity.

In this initial chapter, the reader will be presented to an overview of the cellular components of the immune system and the properties of innate and adaptive responses. It is important to make clear that, for obvious reasons, this text presents a short overview of the knowledge from immunology textbooks but does not replace them. Consequently, it is strongly recommended to the reader that, before reading this book, fundamental concepts on how the immune system works should be attained.

2 Cells of the Immune System

Before further development on the effector mechanisms of the innate and adaptive immune responses, we will start with a general overview on the major cells that constitute the immune system, their production in the bone marrow, release to the blood and, finally, migration to the tissues.

Hematopoiesis—the formation of blood cellular components—begins at early stages of the embryonic development, initially in the yolk sac (mesoblastic phase) and later in the fetal liver and spleen (hepatic phase). Around halfway of the gestation period, the bone marrow gradually assumes the blood production and becomes the exclusive hematopoietic organ by the individual birth (medullary phase), although sites of extramedullary hematopoiesis are recognized in some situations. In the first years after birth, the marrow of essentially every bone produces blood cells. However, along the body's development, this activity becomes restrict to the flat bones (e.g. sternum, pelvis) and some long bones (e.g. femur) [18].

The **hematopoietic stem cells** are multipotent and self-renewing bone marrow cells that generate all the cells in the blood, including those related to the immune system [23]. The hematopoietic stem cells are located in anatomic sites of the bone marrow associated with stromal cells, which do not present hematopoietic activity, but provide the biochemical and the contact-dependent signals required to the proliferation and differentiation of the hematopoietic stem cells. The biochemical signals are represented by growth factors from the **cytokine** family, a group of soluble molecules responsible for the 'communication' of the immune system [11]. In addition to being involved in hematopoiesis, the cytokines have essential roles in the innate and adaptive immune responses including the inflammatory process [20]. Further details will be later discussed in the chapter.

Briefly, the hematopoietic stem cells constantly divide by a process of asymmetric division producing two daughter cells. While one of the daughter cells preserves self-renewal features, the other initiates a differentiation process and develops into a **hematopoietic progenitor**. Along their development, the hematopoietic progenitors

are exposed to certain patterns of cytokines and other growth factors and further differentiate either in **myeloid** or **lymphoid precursors**. The myeloid precursors will give rise to monocytes, neutrophils, eosinophils, basophil, erythrocytes (red blood cells) and megakaryocytes (that will later generate platelets). The lymphoid precursors will give rise to T lymphocytes, B lymphocytes, natural killer (NK) cells and, possibly, some dendritic cells. In a steady-state condition, some of these cells will remain in the blood stream during their whole life (hours to days), unless the organism faces inflammatory and/or infectious challenges; other cells naturally migrate to the different tissues where they will constitute the **resident cells**; and some others will recirculate between blood and lymph and their respective draining organs, spleen and lymph nodes. Each one of these cell populations display a characteristic set of cell surface markers called **cluster of differentiation** (CD) employed to phenotype subpopulations/subsets, differentiation stages, degree of activation and metabolic states [5]. Together, these markers enable us to study different cells in vitro and in vivo and allow comparisons of experimental assays between different research groups.

3 Innate Immunity

A number of physical, chemical and biological barriers constitute the innate immunity and protects our body from noxious agents found in the environment. Among these barriers are the epithelial and mucosal surfaces, the airflow resulting from breathing, coughing and sneezing, the flow of fluids generated by lacrimation and urination, bodily secretions that possess a typical pH and set of enzymes, secretion of mucus and antimicrobial molecules and, finally, the microbiome present in all body's surfaces [21]. Each of these elements has a special role in maintaining the organism's homeostasis, but they are not always sufficient to keep the organism free from aggressive agents. When these initial barriers are "broken" and our body is invaded by a microorganism (pathogenic or not) or another type of aggressor, the innate immune system recognizes conserved structures of these organisms and endogenous molecules produced or released as a result of this invasion. Thus, when **pathogen-associated molecular patterns** (PAMPs) or **damage/danger-associated molecular patterns** (DAMPs) are directly recognized by soluble or cell-associated **pattern recognition receptors** (PRRs), a series of innate immunity events are triggered [26].

Nucleic acids, proteins, lipids and carbohydrates are examples of PAMPs exclusively or differentially expressed by groups of microorganisms that signal their presence to the immune system. DAMPs, on the other hand, are products derived from cellular damage caused by infection or a result of sterile injuries caused by insufficient blood supply and by the action of chemical and physical agents such as toxins, burns, and trauma, among others [3]. In turn, PRRs present in the blood, body fluids and also associated with cells (in vesicles, in the cytoplasm or in the cell surface) recognize these PAMPs and DAMPs and promote their neutralization and removal, either

through uptake by cells known as **phagocytes** or by activation of cytotoxic mechanisms dependent on other cell types and the activation of biochemical cascades. As this is a very broad topic, more details about PAMPs and DAMPs, as well as their molecular recognition by PRRs, can be found in immunology and biochemistry scientific manuscripts [14, 27]. Among the cells of the immune system that participate in innate immunity, we highlight the **alarm cells**, **professional phagocytes** and **cytotoxic cells**. A brief summary of each category is provided below:

- **Alarm cells** are tissue-resident cells capable of directly recognizing aggressor agents and their products. Alarm cells have either preformed mediators or produce them shortly after activation, triggering tissue inflammation. Once the inflammatory process is triggered, changes in the microcirculation occurs, leading to the extravasation of soluble molecules that mediate the initial responses against these agents associated with the recruitment of inflammatory cells from the blood circulation. Tissue-resident mast cells, eosinophils and macrophages are examples of alarm cells.
- **Professional phagocytes** are cells whose main function is to engulf microorganisms, particles and other substances by phagocytosis or endocytosis, leading to their destruction. They may be resident cells (e.g.,: macrophages and dendritic cells) or recruited to the tissues during the inflammatory process (e.g.,: neutrophils and inflammatory monocytes/macrophages).
- **Cytotoxic cells** present cytotoxic granules in their cytoplasm that can be released by exocytosis upon recognition of certain group of ligands in the surface of target cells. Cytotoxic cells are able to kill other cells infected by intracellular bacteria, viruses or cells that undergo malignant transformation and that can originate tumors. The best-known cytotoxic cells of the innate immunity are "natural killer" (NK) cells, but other cells such as macrophages, neutrophils and eosinophils can become cytotoxic in certain situations.

In addition to cellular components, the innate immunity also comprises soluble mediators. The recognition of offending agents can trigger the rapid production and release of **acute-phase proteins**, **inflammatory cytokines**, **chemotactic agents** (molecules that recruit cells to the site) and **vasoactive amines** (substances that activate the vascular endothelium), in addition to the activation of biochemical cascades responsible for blood clotting and the **complement system**, whose components promotes direct lysis of microorganisms.

As said before, once the activation of innate immunity occurs either due to an infectious agent or injury by a physical, chemical or biological agent, its elements will initiate the so-called inflammatory process. Inflammation is a process that involves local vascular changes in the microcirculation such as vasodilation and increased vascular permeability, but that may progress to more serious systemic reactions. The inflammatory process is mediated by the cellular and soluble components described above, whose consequences are represented by five classic signs: **pain** (*dolor*), **heat** (*calor*), **redness** (*rubor*), **swelling** (edema or *tumor*) and **loss of function** (*functio laesa*) [17]. In this microenvironment, the elements of innate immunity work together

to contain/destroy the aggressor agent and also initiate the activation of adaptive immunity, discussed below.

For space limitations, the inflammatory process will not be described in further details, but the topic can be found in immunology, pharmacology and pathology textbooks. In addition, many immunometabolic aspects of the innate immunity and inflammation will be approached in the next chapters.

4 Adaptive Immunity

Invading microorganisms have varied strategies for evading immune responses. In many situations, the cells and soluble factors of innate immunity are not efficient in eradicating them from our body. In the first days following infection, the elements of innate immunity attempt to control the growth and spread of microorganisms. Meanwhile, the cells of adaptive immunity need to be activated so that they can fully exert their effector functions as soon as they are ready for that. The cells of the adaptive immunity are collectively called **lymphocytes** and will be responsible, either directly or indirectly, for the effector mechanisms against the different aggressor agents. There are two major populations of lymphocytes, known as **T cells** (or T lymphocytes) and **B cells** (or B lymphocytes). T cells were named after the discovery that the **thymus** is the organ responsible for their maturation and selection ("T" from thymus) [15]. Several subpopulations of T cells are currently known that, together, represent the **cell-mediated adaptive responses** described in more details later. Likewise, B cells were described following the observation that chickens whose bursa of Fabricius had been removed were not able to produce **antibodies** [4]. The importance of antibodies as the effector molecules of **humoral adaptive immune responses** was already known, but the role of B cells in their production was uncovered by these studies ("B" from bursa). The structure and role of antibodies will be also discussed below. Humans do not have bursa of Fabricius, and our B cells are produced, matured and selected in the bone marrow (although the spleen also participates of the process). Because the bone marrow, bursa of Fabricius and the thymus are the primary sites responsible for the production and development of lymphocytes, these organs are collectively called **primary** (or **central**) **lymphoid organs**.

Lymphocytes express receptors with high specificity and accessory molecules on their surface that allow their activation and function as markers used by researchers to characterize different lymphocyte populations and subpopulations. The lymphocyte receptors are able to recognize a very large number of microbial and non-microbial substances collectively called **antigens**. For example, **T cell receptors** (TCRs) recognize peptides resulting from the processing of extracellular or intracellular proteins, derived from our own organism or from microorganisms and their products. The processing of such proteins takes place in specialized cells known as professional **antigen-presenting cells** (APCs) which are capable of capturing, degrading and displaying fragments of these proteins on the surface linked to molecules of the **major histocompatibility complex** (MHC). There are three types of professional

APC in our organism: dendritic cells, macrophages and B cells. In summary, the T lymphocyte can only be activated when its TCR (together with accessory molecules) recognizes peptides displayed by the MHC in the APC surface [10]. On the other hand, **B cell receptors** (BCRs) are able to directly recognize antigens of different biochemical nature such as proteins, carbohydrates, lipids and nucleic acids, among others. The BCR is an antibody linked to the membrane of B cells that binds cell-associated or soluble antigens present in the extracellular *milieu.* The binding of antigens by the BCR delivers activation signals to B cells which, in turn, produce and secrete large quantities of the antibody in a soluble form.

Unlike innate immunity cells, which individually express a large number of PRRs (thus being able to recognize many different PAMP and DAMP at the same time), lymphocytes have their receptors distributed clonally. That is, a lymphocyte expresses hundreds of thousands of receptors on its surface, but in an individual cell (**clone**), all of these receptors recognize the same antigen. Therefore, although an individual has millions of lymphocytes in its circulation, only a few clones will be specific for a given antigen. The sum of all antigens capable of being recognized by lymphocyte clones present in our body constitutes an individual's **immune repertoire** [13]. As a consequence of the small number of specific clones for each antigen, once activated, lymphocytes must enter a process of **clonal expansion** in order to reach a critical number of cells capable of handling each antigen. Because it takes 8–12 h for a lymphocyte clone to complete each division cycle, a few days (typically 5–12 days) are needed until the role of adaptive immunity in an infection is perceived. However, the great advantage of the adaptive immunity is the generation of **immunological memory**, that is, once activated, part of the lymphocytes will become memory cells and will survive for months or years, even after the pathogen/antigen elimination [16, 25]. In subsequent contacts with the same agent, these cells will be present in greater numbers and will be activated more quickly, ensuring full and fast protection to our organism. For that reason, most pathogens only cause disease the first time they come into contact with our organism. This knowledge is widely explored in the development of **vaccines**, which are safe preparations capable of mimicking an infection, leading to the development of protective immune responses and immunological memory when we come into contact with the real pathogen [19].

The activation of lymphocytes is a complex process and results from the recognition of antigens by TCR or BCR, triggering intracellular signaling cascades responsible for the transduction of activation signals. Together, these signals induce activation of protein kinases, phosphorylation of biochemical substrates, increase in cytoplasmic calcium and activation of nuclear transcription factors that lead to cell proliferation and synthesis of molecules responsible for the effector mechanisms of T cells and B cells. Such cascades will be covered in this book, when appropriate, in the context of immunometabolism.

4.1 Cell-Mediated Adaptive Immune Responses

As mentioned before, **cell-mediated adaptive immune responses** are initiated when APCs present at the site of an infection (usually immature dendritic cells in the tissues), capture microorganisms or their products and process protein antigens for presentation by MHC molecules. The whole process is accompanied by **dendritic cell maturation**, leading to phenotypic changes and culminating in morphological and physiological changes in these cells, followed by migration to draining lymph nodes [2]. In cases where the infection occurs directly in the bloodstream, the microorganisms and their products will be drained by the spleen, where antigen processing and presentation will take place by splenic APCs. Lymph nodes, spleen and other organized lymphoid clusters known as mucosa-associated lymphoid tissue (MALT), bronchus-, larynx- and nose-associated lymphoid tissue (BALT, LALT, NALT) and gut-associated lymphoid tissue (GALT), are collectively known as **peripheral** (or **secondary**) **lymphoid organs**, and represent the places where lymphocytes accumulate during their recirculation in the body and where the activation of these cells occurs. In these lymphoid organs and tissues, newly arrived and already mature dendritic cells express high levels of MHC molecules containing peptide fragments derived from microbial proteins that will bind to TCRs, representing the **first signal of activation**. Mature dendritic cells also express costimulatory molecules that, upon interaction with their respective ligands present in T cells, will represent the **second signal of activation**. Finally, APC/T lymphocyte interactions occur in the presence of cytokines that some authors consider the **third signal of activation** [7]. Together, these molecular interactions will induce T cell proliferation and differentiation into effector cells. Once differentiated, the lymphocytes will leave the peripheral lymphoid organs/tissues and migrate to the site where the infection was originally established, thus performing its effector functions. As mentioned before, some of these cells will differentiate into memory cells.

When the antigens processed by dendritic cells are exogenous/extracellular in nature (e.g. captured by phagocytosis/endocytosis), they will be displayed by MHC class II molecules and the complex will interact with T cells that express a co-receptor called CD4. These $CD4^+$ T cells are also known as **T helper** (**Th**) cells. During the final stage of activation Th cells will differentiate into cytokine-producing cells that receive different terminologies according to the cytokine profile produced (e.g. Th1, Th2, Th17, Tfh) [8]. In turn, the cytokines produced by T cells will activate/modulate effector cells such as neutrophils, eosinophils, macrophages and B lymphocytes among others, responsible for battling and eradicating the microorganism/offending agent—toxins, poisons, bacteria, viruses, fungi, helminths, protozoa, and others. During this process, **regulatory T cells** (**Treg**) are also generated, whose role is to maintain tolerance and regulate immune responses, preventing them from becoming exacerbated or harmful [22]. On the other hand, when the antigens processed by dendritic cells are endogenous/intracellular in nature (e.g. products of viral infections or intracellular bacteria), they will be displayed by MHC class I molecules and the complex will interact with T cells that express a co-receptor called CD8. These $CD8^+$

T cells are also known as **cytotoxic** or **cytolytic T cells** (**Tc**) [9]. After activation, Tc lymphocytes will differentiate into cells capable of killing other cells infected by intracellular bacteria or viruses.

4.2 Humoral Adaptive Immune Responses

Adaptive humoral immune responses are represented by antibodies, also called immunoglobulins or gammaglobulins. Antibodies are glycoproteins produced by B cells that are capable of recognizing and binding to antigens of different biochemical nature (proteins, carbohydrates, lipids, nucleic acids, among others). In mature B cells that have never found the antigen (naïve cells), antibodies are present on the membrane surface and act as BCR for these cells. A foreign invading agent and/or its antigens will be carried to secondary lymphoid organs and will be recognized by the BCR of some B cell clones that are constantly recirculating among these organs. The BCR/antigen interaction can directly activate B cells and induce their proliferation (**clonal expansion**) and differentiation into **plasma cells**, capable of secreting large amounts of that specific antibody. These antibodies will circulate in the body fluids or will be transported to mucosal surfaces, where they remain available to bind any suitable antigen for varied periods of time. This interaction, known as the **antigen–antibody reaction**, will be essential to eliminate the offending agent directly (e.g. neutralizing toxins, bacteria, viruses, etc.) or indirectly (e.g. increasing phagocytosis, activating phagocytes and the complement system, promoting antibody-dependent cytotoxicity), as described below. It is worth mentioning that in some situations B cell can also work as APCs and be activated with the help of **T follicular helper** (**Tfh**) cells, the so-called **T-dependent** activation [6]. In other situations, the B cell can be activated directly, without the participation of Th cells, the so-called **T-independent** activation [1]. The requirements for each type of activation will not be covered in this chapter, but will depend on the population of B cells, the nature and amount of the antigen, and other factors present in the microenvironment during activation. In addition, only T-dependent activation will be able to generate memory B cells.

Briefly, the antibody consists of two identical larger chains (**heavy chains**) and two smaller identical chains (**light chains**). The heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain also by disulfide bonds. Both heavy and light chains are constituted of domains: regions with a relatively conserved sequences of amino acids that repeats throughout the molecule. The heavy chains have 4 or 5 domains each (one called a variable domain and the others called constant domains), while the light chains always have 2 domains (a variable domain and a constant domain). In the region where the two domains of each light chain are paired with the respective domains of the heavy chain, there is a portion that recognizes the antigen called, therefore, **antigen-binding fragment** (**Fab**). An antibody monomer has two Fab. At the tip of each Fab there is one region formed by the outermost portions of the variable domains of heavy chain and light chain, where the interaction with the antigen effectively occurs. Therefore, this

portion is called **antigen-binding region** and concentrates most of the variability of each antibody molecule. On the other hand, the region where the two heavy chains are paired is responsible for the effector functions of the antibody. Because of its biochemical characteristics, it is called a **crystallizable fragment** (**Fc**). The region that separates the Fc from the two Fabs present in a monomeric antibody molecule is called **hinge** and gives flexibility to the molecule, thus allowing a monomeric antibody to bind to two identical antigens at the same time and at different angles. Some classes of antibodies can form dimers, trimers, pentamers and hexamers, so that they can bind multiple identical antigens at the same time (four, six, ten and twelve, respectively).

It is important to note that "antibody" is a generic nomenclature that actually represents a group of related molecules that have some structural and functional differences. According to these characteristics, antibodies are grouped into five **isotypes** (or **classes**) that in humans and other mammals: IgM, IgD, IgG, IgA and IgE. In some cases, there are subclasses (e.g. IgG1, IgG2, IgG3 and IgG4 in humans) that are differentially expressed depending on the type of immune responses triggered by a given infection. In all cases, antibodies bind to antigens in a non-covalent and reversible manner, through a series of biochemical interactions that include electrostatic forces, hydrogen bonds, van der Waals forces and hydrophobic interactions. Together, these forces define the affinity of an antibody binding site to the antigen, and the overall strength of all binding sites is called avidity (as we have seen, an antibody has at least two antigen binding sites). This bond is strong enough to ensure that, once bound, the antibody will hardly detach from its respective antigen. This ensures that antibody's effector functions can be adequately performed, including neutralization of toxins, viruses and bacteria, activation of the complement system, antibody-dependent cell cytotoxicity and opsonization (facilitation of phagocytosis).

In addition to essential effector molecules against a variety of infections, antibodies are also important for assessing the immunity developed after a vaccination and for diagnosing a range of diseases. As antibodies are present in the circulation, the collection of a small amount of blood makes it possible to assess in a patient's serum or plasma whether he/she has already been exposed to a certain antigen. In certain cases, it is even possible to determine whether the clinical condition is due to an acute or primary infection or whether the infection has already become chronic or recurrent, based both on the isotype/class and the antibody title (an indirect way of assessing the amount of antibodies present for a given antigen). On the other hand, the absence of one or more classes of antibodies may be indicative of **immunodeficiencies**.

Antibodies are among the most studied molecules in the biochemical field both structurally and functionally. This knowledge allowed the development of serum therapy over a century ago, still used nowadays to treat the bites of venomous animals such as snakes, spiders and scorpions; bacterial-derived toxins such as diphtheria and tetanus toxins; and even active infections such as Ebola and COVID-19. Advances in cell culture, establishment of tumoral cell lines and development of molecular biology techniques allowed the creation of **monoclonal antibodies**—identical antibodies with unique and known specificity—that can be produced in industrial scale

for research and clinical use. The monoclonal antibodies are the major representatives of the so-called **immunobiologicals**, molecules of biological origin capable of modulating the immune system and used as a treatment of several pathologies of microbial origin, cancer and autoimmune diseases. Immunobiologicals are already a reality for a number of clinical conditions and are among the blockbuster drugs for the pharmaceutical industry. However, its price is still high, which limits its access to most of the population.

5 Final Remarks

Now that you reviewed how immune cells are generated, how foreign agents are recognized, and how the immune responses that will fight them are initiated, the next chapters will present deeper aspects of these topics in a context of immunometabolism. You will have the opportunity to learn more details about metabolic pathways and mitochondrial physiology in health and disease. In addition, up to date knowledge on immunometabolism will be reviewed in a number of clinical conditions such as obesity, cancer, autoimmune diseases, organ transplantation and inflammatory/infectious diseases. Finally, interfaces between immunometabolism and microbiota, physical exercise, immunotherapies and translational medicine will be also approached.

References

1. Allman D, Wilmore JR, Gaudette BT (2019) The continuing story of T-cell independent antibodies. Immunol Rev 288(1):128–135
2. Austyn JM (2016) Dendritic cells in the immune system-history, lineages, tissues, tolerance, and immunity. Microbiol Spectr 4(6)
3. Bianchi ME (2007) DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 81(1):1–5
4. Cooper MD (2015) The early history of B cells. Nat Rev Immunol 15(3):191–197
5. Cruse JM, Lewis RE, Wang H (2004) Cluster of differentiation (CD) antigens. Academic Press, Immunology Guidebook, pp 47–124
6. Crotty S (2015) A brief history of T cell help to B cells. Nat Rev Immunol 15(3):185–189
7. Curtsinger JM, Mescher MF (2010) Inflammatory cytokines as a third signal for T cell activation. Curr Opin Immunol 22(3):333–340
8. Gagliani N, Huber S (2017) Basic aspects of T helper cell differentiation. Methods Mol Biol 1514:19–30
9. Golstein P, Griffiths GM (2018) An early history of T cell-mediated cytotoxicity. Nat Rev Immunol 18(8):527–535
10. La Gruta NL, Gras S, Daley SR, Thomas PG, Rossjohn J (2018) Understanding the drivers of MHC restriction of T cell receptors. Nat Rev Immunol 18(7):467–478
11. Lin JX, Leonard WJ (2019) Fine-Tuning Cytokine Signals. Annu Rev Immunol 26(37):295–324
12. Litman GW, Rast JP, Fugmann SD (2010) The origins of vertebrate adaptive immunity. Nat Rev Immunol 10(8):543–553

13. Liu X, Wu J (2018) History, applications, and challenges of immune repertoire research. Cell Biol Toxicol 34(6):441–457
14. Medzhitov R (2009) Approaching the asymptote: 20 years later. Immunity 30(6):766–775
15. Miller JF (2004) Events that led to the discovery of T-cell development and function—a personal recollection. Tissue Antigens 63(6):509–517
16. Mueller SN, Gebhardt T, Carbone FR, Heath WR (2013) Memory T cell subsets, migration patterns, and tissue residence. Annu Rev Immunol 31:137–161
17. Nathan C (2002) Points of control in inflammation. Nature 420(6917):846–852
18. Orkin SH, Zon LI (2008) Hematopoiesis: an evolving paradigm for stem cell biology. Cell 132(4):631–644
19. Pollard AJ, Bijker EM (2021) A guide to vaccinology: from basic principles to new developments. Nat Rev Immunol 21(2):83–100
20. Ramani T, Auletta CS, Weinstock D, Mounho-Zamora B, Ryan PC, Salcedo TW, Bannish G (2015) Int J Cytokines: the good, the bad, and the deadly. Toxicol 34(4):355–65
21. Riera Romo M, Pérez-Martínez D, Castillo FC (2016) Innate immunity in vertebrates: an overview. Immunology 148(2):125–139
22. Sakaguchi S (2011) Regulatory T cells: history and perspective. Methods Mol Biol 707:3–17
23. Sawai CM, Babovic S, Upadhaya S, Knapp DJHF, Lavin Y, Lau CM, Goloborodko A, Feng J, Fujisaki J, Ding L, Mirny LA, Merad M, Eaves CJ, Reizis B (2016) Hematopoietic stem cells are the major source of multilineage hematopoiesis in adult animals. Immunity 45(3):597–609
24. Thaiss CA, Levy M, Itav S, Elinav E (2016) Integration of innate immune signaling. Trends Immunol 37(2):84–101
25. Weisel F, Shlomchik M (2017) Memory B cells of mice and humans. Annu Rev Immunol 26(35):255–284
26. Yin Q, Fu TM, Li J, Wu H (2015) Structural biology of innate immunity. Annu Rev Immunol 33:393–416
27. Zindel J, Kubes P (2020) DAMPs, PAMPs, and LAMPs in immunity and sterile inflammation. Annu Rev Pathol 24(15):493–518