Immune system – Part I

Fundamentals of innate immunity with emphasis on molecular and cellular mechanisms of inflammatory response

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ABSTRACT

The immune system consists of an intricate network of organs, cells, and molecules responsible for maintaining the body’s homeostasis and responding to aggression in general. Innate immunity operates in conjunction with adaptive immunity and is characterized by rapid response to aggression, regardless of previous stimulus, being the organism first line of defense. Its mechanisms include physical, chemical and biological barriers, cellular components, as well as soluble molecules. The organism first line of defense against tissue damage involves several steps closely integrated and constituted by different components of this system. The aim of this review is to restore the foundations of this response, which has high complexity and consists of several components that converge to articulate the development of adaptive immune response. We selected some of the following steps to review: perception and molecular recognition of aggressive agents; activation of intracellular pathways, which result in vascular and tissue changes; production of a myriad of mediators with local and systemic effects on cell activation and proliferation, synthesis of new products involved in the chemotraction and migration of cells specialized in destruction and removal of offending agent; and finally, tissue recovery with restoration of functional tissue or organ.

Keywords: innate immunity, inflammation, autoimmunity, PAMPs, Toll-like receptors.

INTRODUCTION

The immune function has been conceptually divided into innate and adaptive immunity. Innate immunity represents a rapid and stereotyped response to a large but limited number of stimuli. It is represented by physical, chemical, and biological barriers, specialized cells and soluble molecules, present in all individuals, irrespective of previous contact with offending agents or immunogens, and does not change qualitatively or quantitatively after contact.1 The main effector cells of innate immunity are macrophages, neutrophils, dendritic cells, and natural killer (NK) cells (Table 1). Phagocytosis, release of inflammatory mediators, activation of complement system proteins, as well as synthesis of acute phase proteins, cytokines and chemokines are the main mechanisms in innate immunity. These mechanisms are activated by specific stimuli, represented by molecular structures of ubiquitous occurrence in microorganisms, but not in human species. Molecules commonly found on the surface of microorganisms, such as lipopolysaccharides, mannose
and teichoic acids constitute pathogen-associated molecular patterns (PAMPs) and activate the innate immune response by interaction with different receptors known as pattern recognition receptors (PRR), among which is the family of Toll-like receptors (TLRs). This interaction is similar to the complementarity between antigen and antibody or between antigen and T-cell receptor (TCR), but in this case, there is no diversity or adaptive capacity to generate new receptors or recognition of new molecular patterns than those already programmed in genetic code.

Among the various PRRs involved in opsonization, complement activation, and phagocytosis, the TLRs are distinguished by their central role in binding to pathogens and initiating the inflammatory response. These receptors are present mainly on macrophages, neutrophils, and dendritic cells (DCs). Currently, eleven different TLRs have been identified, some located in the cell membrane, others inside the cells (Figure 1). Other receptors present in phagocytes with important role in immune response are those for fractions of complement, cytokines, interleukins, and immunoglobulins (FcγR type).

Phagocytosis begins with adhesion of the phagocyte surface receptors to the pathogen, which then is internalized into vesicles called phagosomes. Inside the phagocyte, the phagosome fuses to lysosomes, whose contents are released with consequent digestion and pathogen elimination. Changes in the oxidase’s gene system components present in phagolysosome membrane lead to disability in respiratory burst and generation of reactive oxygen species (ROS). The absence of ROS determines serious deficiency in the destructive capacity of phagocytes, being responsible for a significant primary immunodeficiency called chronic granulomatous disease.

Unlike innate response, adaptive or acquired immune response depends on activation of specialized cells (the lymphocytes) and soluble molecules produced by lymphocytes (Table 1). The main features of acquired response are: specificity and diversity of recognition, memory, specialized response, self-restraint, and tolerance to components of the organism itself. Although the main cells involved in acquired immune response are lymphocytes, antigen presenting cells (APCs) play a key role in its activation, presenting antigens associated with molecules of the major histocompatibility complex (MHC) to T lymphocyte (TL). Figure 2 illustrates the various cells that comprise the immune system.

**DENDRITIC CELLS**

Dendritic cells, specialized in capturing and presenting antigens to lymphocytes, are considered a bridge between innate and adaptive immunity because they are attracted and activated by elements of innate response and permit TL sensibilization of adaptive immune response. Dendritic cells reside in peripheral tissues, such as skin, liver, and intestine, where they capture antigens and become activated and migrate to regional lymph nodes, in which they process and present protein antigen or lipid to TLs. Immature DCs are highly efficient in capturing antigens, while mature DCs are very efficient in presenting antigens. The captured antigens are processed inside the cell and presented on its surface, bound to MHC molecules. Generally, protein antigens are presented by MHCs classical molecules (class I and II) that stimulate LTαβ. Lipid antigens...
are presented by MHCs non-classical molecules as CD1 and stimulate primarily LTγδ and NK/T.

During their lifetime, the immature DCs migrate from bone marrow into bloodstream, reaching peripheral tissues as skin, where they become residents (Langerhans cells). A curious aspect is that DCs are the first cells to arrive at a site of infection, preceding even the neutrophils. After contact with antigen, DCs become activated and migrate through lymphatic vessels to secondary lymphoid organs (Figure 3). They can receive signals from mature NK, NK/T, and TL cells, and proinflammatory molecules such as cytokines, prostaglandins, interferons, and PAMPs. DCs retain antigen in lymphoid organs for extended periods, which may contribute to immunological memory. These cells orchestrate the migration of other types of immune cells within the lymph nodes via secretion of chemokines and regulate the differentiation, maturation, and function of TL in a contact-dependent mode and by secretion of soluble factors. Therefore, DCs are essential for the initiation and coordination of the acquired immune response.

There are two pathways of DCs differentiation from a common progenitor. The myeloid pathway generates myeloid DCs (mDCs), among which there are the Langerhans cells, the main DCs in skin, and the interstitial DCs found in other tissues. The other pathway of differentiation generates plasmacytoid DCs (pDCs), which predominate in the peripheral blood and secrete large amounts of type I interferon (IFN-α/β) in the presence of viral infections. The pDCs have receptors capable
of responding to RNA (TLRs 7 and 8) and DNA (TLR9), whereas mDCs preferentially express surface receptors for PAMPs, such as peptidoglycan (TLR2) and lipopolysaccharide (TLR4).9

DCs are crucial for determining the activation and type of immunity mediated by TLs. In general, immature DCs are tolerogenic, while mature DCs are immunostimulatory. However, in some contexts, mature DCs can expand the population ofTLs regulators. Induction of tolerance or immune response depends on the set of signals received by DCs, such as activation ofTLRs and cytokines present in the environment.10

DCs can coordinate LBs response via TL activation or directly by soluble substances such as INF-α.7

**NEUTROPHILS**

Neutrophils are the most abundant leukocytes in peripheral blood, with an important role in the early stages of inflammatory reaction and sensitive to chemotactic agents, such as cleavage products of complement fractions (C3a and C5a) and substances released by mast cells and basophils. They are among the first cells to migrate from vessels to tissues attracted by chemokines, such as IL-8, and are activated by various stimuli, such as bacterial products, complement proteins (C5a), immune complex (IC), chemokines, and cytokines.

The neutrophil phagocytic capacity is stimulated by binding of its receptors for opsonins, IgG-Fc, C3b, and TLRs. These cells also undergo degranulation, releasing three classes of granules in the extracellular environment:

- Primary or azurophilic granules that contain important mediators, such as myeloperoxidase, defensins, neutrophil elastase, permeability-increasing protein, and bacterial cathespin G.
- Secondary granules with components specifically secreted by neutrophils, with lactoferrin is a prime example.
- Tertiary granules with cathespins and gelatinases as main proteins.

Recent studies have shown that neutrophils can also generate the so-called neutrophil extracellular traps (NETs) formed by granule substances and nuclear components capable of calling off the virulence factors and destroying extracellular bacteria. The NETs are present in large quantity in inflammatory sites, acting directly on microorganisms and also serving as a physical barrier that prevents spreading.11

Under normal conditions, neutrophils are cleared from the circulation and inflamed tissues by apoptosis. Disturbances in these cells apoptosis have been associated with several autoimmune conditions, especially SLE, as circulating apoptotic debris containing nuclear material could lead to the induction of a huge variety of autoantibodies.11

**MACROPHAGES**

Monocytes constitute 3-8% of circulating leukocytes and, in connective tissue or parenchyma of organs, give rise to macrophages and myeloid dendritic cells. Monocytes and macrophages are efficient phagocytes, engulfing pathogens and cellular debris. Unlike neutrophils, macrophages can remain in tissue for months to years, acting as true sentinels. Besides having a role in innate immunity, macrophages process and present antigens via MHC molecules, thus stimulating the response mediated by TL.4

Recently, the existence of three subpopulations of macrophages was proposed: activated, tissue repair, and regulator macrophages. The first would be the classic macrophages with tumoricidal and microbicidal activity, which secrete large amounts of proinflammatory mediators and cytokines, present antigens to TLs, and are involved in cellular immune response. The second type, activated by IL-4, would be primarily involved in tissue repair by stimulating fibroblasts and promoting extracellular matrix deposition. The third type would exert regulatory activity through release of IL-10, an anti-inflammatory cytokine.13

In inflammation, macrophages act as APCs, potentiating the activation of TL and LB by the expression of coestimulatory molecules, and release proinflammatory cytokines, such as IL-1, IL-6, IL-12, TNF-α, and chemokines. They also produce reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, hydrogen peroxide (H2O2), and reactive nitrogen intermediates whose main representative is nitric oxide (NO). NO is produced by inducible nitric oxide synthase, iNOS, absent in resting macrophages, but induced by TLRs activation in response to PAMPs, especially in the presence of INF-γ.4

Some microorganisms, like *Mycobacterium tuberculosis*, are resistant to the microbicidal action and remain viable for a long time in macrophages’ phagosomes. These macrophages become large and multinucleated (giant cells) and, together with lymphocytes and fibroblasts that accumulate around them, form granulomas, which are the body’s attempt to prevent the spread of the pathogen.

**NATURAL KILLER CELLS**

Natural killer cells (NK) originate in bone marrow from a common progenitor to TLs, constituting 5% to 20% of blood...
mononuclear cells. They are an important line of nonspecific defense, recognizing and lysing cells infected by viruses, bacteria and protozoa, as well as tumor cells. Furthermore, they recruit neutrophils and macrophages, activate DCs and T and B lymphocytes.14

The expansion and activation of NKs are stimulated by IL-15, produced by macrophages, and IL-12, potent inducer of IFN-γ and cytolytic action. Once activated, the NKs lyse infected and tumoral cells and secrete proinflammatory cytokines (IL-1, IL-2, and especially IFN-γ).14

The cytolysis mediated by NKs occurs through the action of the enzymes perforins, which create pores in membrane of target cells, and granzymes, which penetrate into cells and trigger cell death by apoptosis. NK cells have activation and inhibition receptors, and the balance between the signals generated by these receptors determines NK activation. One class of receptors belongs to the immunoglobulin superfamily (KIR), while the other belongs to the family of C-type lectins. In humans, there are 14 KIRs, eight activators and six inhibitors.15 The inhibitory receptors recognize self MHC class I molecules, expressed on the surface of all nucleated cells. In general, there is dominance of inhibitory receptors, preventing lysis of host’s normal cells that express MHC class I. Infected cells, especially by viruses, and tumor cells often have low expression of MHC class I proteins, becoming vulnerable to the action of NK (Figure 4).15 The tumoricidal capacity of NK is increased by cytokines, such as interferons and interleukins (IL-1, IL-2 and IL-12). Another effector action of NK is the destruction of cells coated with IgG, via Fc receptors (FcγRIII or CD16), by a mechanism of antibody-dependent cellular cytotoxicity (ADCC).14

MAST CELLS

Mast cells are derived from CD34+ hematopoietic progenitors in bone marrow and, in general, are not found in the circulation. From bone marrow, the progenitors migrate to peripheral tissues as immature cells and differentiate in situ according to the particular characteristics of the microenvironment.16,17

Mature mast cells are distributed strategically along the blood vessels, nerves, and under the epithelium of skin and mucous membranes; they are particularly abundant in areas of environment contact and play a key role in acute inflammatory reactions.18 Mast cells have surface receptors of high affinity, FcεRI, bound to IgE molecules, and are activated by multivalent antigen recognition by IgE. Stimuli such as products of complement activation, basic substances, including some animals’ poisons, certain neupeptides, and several physical agents (mechanical trauma, heat, and cold) can activate mast cells independently of IgE binding. The binding of bacterial components to TLRs 1, 2, 4, and 6, and other specific receptors such as CD48, also activates mast cells, leading to mediators’ release.

The classic example of mast cells involvement in inflammatory processes are the reactions in which they, together with its circulating equivalent, basophil, in contact with the allergen trigger a type I hypersensitivity reaction via FcεRI activation. After the stimulus, degranulation and release of preformed mediators occur, followed by the release of newly formed mediators. Preformed mediators include vasoactive amines, proteases, heparin, IL-4, TNF-α, and GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor). The mediators formed following activation include platelet

Figure 4
Function of receptor activation (ITAM) and inhibition (ITIM) in the physiology of NK cells. (A) Interaction of NK cells with a normal body cell expressing MHC class I, with consequent inhibition of NK cytolysis dependent induction. (B) Interaction of NK cells with virus-infected cell, with consequent MHC class I loss of expression, which results in activation of NK cells with concomitant release of lethal products.
activating factor (PAF), arachidonic acid derivatives, and a series of cytokines. The release of these mediators induces inflammatory cell migration (neutrophils and macrophages), increased vascular permeability, mucus secretion, increased gastrointestinal motility, and bronchoconstriction, which are the signs and symptoms of allergy and anaphylaxis.

Chronic Idiopathic urticaria is mainly caused by degranulation of mast cells, and in 25% to 50% of cases autoantibodies directed against the FcεRIα receptors are found and, less frequently, against IgE itself. These autoantibodies cause histamine release and characterize the chronic autoimmune urticaria, with clinical and histological features similar to those found in a late-phase reaction.

There is experimental evidence of mast cells involvement in cardiovascular diseases, neoplastic diseases, parasitic and bacterial infections, fibrotic diseases, and autoimmune diseases. Several histological studies have reported the presence of mast cells in normal human synovium and expansion of this population in rheumatoid arthritis, gout, osteoarthritis, among others. Effector functions of mast cells in synovia suggest their involvement in leukocyte recruitment, fibroblast activation and hyperplasia, angiogenesis, and destruction of cartilage and bone. They also participate in joint destruction by inducing fibroblasts and chondrocytes to secrete matrix metalloproteinases and promoting osteoclast differentiation. In fact, the involvement of mast cells with chemotactic activity has been reported in various autoimmune clinical conditions, including rheumatoid arthritis, Sjögren’s syndrome, systemic sclerosis, autoimmune thyroid diseases, chronic urticaria, pemphigus, and atherosclerosis.

**BASOPHILS**

Basophils are granulocytes derived from progenitors in bone marrow, where they mature and make up less than 1% of peripheral blood leukocytes. Although not normally present in tissues, they can be recruited to inflammatory sites, together with eosinophils. The granules found in basophils have mediators similar to those of mast cells. Basophils also express FcεRI, bind IgE, and are activated by IgE-antigen complexes and may contribute to immediate hypersensitivity reactions.

**EOSINOPHILS**

Granulocytes and eosinophils are important infection-fighting cells, and their antiparasitic action (helminths) is one of the most powerful and effective. They are also important in allergic reactions and asthma. Eosinophils develop in the bone marrow, producing and storing various secondary proteolytic granules before leaving the marrow. After maturation, they circulate through the bloodstream in small amounts and can be found in greater numbers in mucosal regions, such as gastrointestinal, respiratory, and genitourinary tracts. Eosinophils are recruited to sites of parasitic infections and allergic reactions by adhesion molecules and chemokines. They fight parasitic infections by antibody-dependent cell-mediated cytotoxicity, with FcεRI receptor participation. During this process, they adhere to pathogens coated with IgE (or IgA) and release their granular content after FcεRI receptors bind to IgE bound to the target antigen. Once activated, eosinophils induce inflammation through production and release of eosinophilic cationic content of granules. The main components of these granules are: major basic protein, eosinophil cationic protein, eosinophil derived neurotoxin, and eosinophil peroxidase, which have great potential cytotoxicity on parasites, but also can cause tissue injury. Eosinophil cationic protein and neurotoxin are ribonucleases with antiviral properties. The major basic protein presents toxicity to parasites, induces degranulation of mast cells and basophils, and activates the synthesis of remodeling factors by epithelial cells. Eosinophil cationic protein creates pores in target-cell membrane, allowing the entry of other cytotoxic molecules; inhibits TL proliferation; suppresses antibody production by LB; induces degranulation of mast cells; and stimulates secretion of glucosaminoglycans by fibroblasts.

Eosinophil peroxidase forms ROS and NO, promoting oxidative stress in target-cell and causing cell death by apoptosis and necrosis. Other effector mechanisms that contribute to the inflammatory process include production of a variety of cytokines, such as IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-13, and TNF-α, and release of proinflammatory lipid mediators, such as leukotrienes (LTC4, LTD4, LTE4), and prostaglandins (PGE2). Elastase enzymes and the growth factor TGF-β, growth factor derived from platelets (PDGF), and endothelial vessels growth factor (VEGF) contributes to tissue remodeling.

**THE COMPLEMENT SYSTEM**

Complement system (CS) consists of a family of more than 20 plasma glycoproteins, synthesized in the liver, but also by macrophages and fibroblasts. Each SC activated component acquires proteolytic activity activating the next elements in cascade. Throughout the process, there is the production of several mediators that alter vascular permeability and contribute to the development of inflammatory response.
Finally, there is formation of membrane attack complex (MAC), which promotes osmotic lysis of target-cell, favoring the elimination of the infectious agent.4

There are three forms of CS activation: classical, alternative, and via mannose-binding lectin (MBL). The activation of these pathways contributes to the integration of effector mechanisms of innate and adaptive immunity (Figure 5). In the innate immune response, pathogens that invade the organism encounter soluble substances of innate immune response, such as CS proteins, C-reactive protein, and others. In adaptive immunity, CS is activated by binding of preformed antibodies to pathogen or antigen (immune complex).26 Lectin pathway begins by recognizing mannose on microorganism surface by MBL bound to MASP1 and MASP2 serine proteases. Activation of these proteases results in the breakdown of CS components C2 and C4 into smaller fragments (C4a and C2b) and larger fragments (C4b and C2a). C4bC2a complex is the C3 convertase of the classical pathway, which cleaves C3 into soluble C3a and C3b, which in turn binds to C4bC2a at the surface of microorganism. The C4bC2aC3b complex, called C5 convertase, cleaves the C5 component, following on this pathway and culminating in the formation of MAC. The classical pathway resembles the lectin pathway and is initiated by the binding of C1q component to two molecules of IgG or to one molecule of IgM, complexed with the target antigen (immune complexes). This binding activates the proteases R (C1r) and S (C1s) associated with C1q, cleaving components C2 and C4 and following the pathway, as described. Because the classical pathway depends on the prior production of specific antibodies attached to the surface of pathogens, it is associated with specific humoral immune response.26

The alternative pathway begins with the spontaneous rupture of the C3 component into C3a and C3b fragments (Figure 5). A thioester binding in fragment C3b is exposed with this cleavage, which allows their covalent binding to the surface of invading microorganisms. If there is no binding of C3b component, thioesters binding site is rapidly hydrolyzed and the fragment is inactivated. The binding of C3b enables binding to Factor B, which is then cleaved into fragments Ba and Bb by Factor D. The C3bBb complex (alternative pathway C3 convertase) cleaves more C3 molecules and remains on the surface. This complex is stabilized by properdin (Factor P), amplifying the breakdown of C3. C3bBb component cleaves C3, generating C3bBbC3b, a protease able to cleave C5, the last step of the alternative pathway.26 Lectins pathways, classic and alternative, have in common the formation of C5 convertase, which promotes cleavage of C5 component and generates fragments C5a and C5b. The binding of C5b to pathogen surface initiates the formation of membrane attack complex by binding successive components C6 and C7 in the lipid bilayer of the cell membrane. The C5b,6,7 complex allows the binding of C8 component and, finally, there is polymerization of C9 crossing the lipid bilayer and promoting osmotic lysis of infectious agent.

Smaller fragments released during the cascade have important biological effects. C2a and C4a are related to changes in vascular permeability; Bb is related to activation of macrophages; C3a, C4a, and C5a induce activation of mast cells and neutrophils; and C5a stimulates motility and adhesion of neutrophils to inflammatory foci. The fragments C3b and C4b function as opsonins, enhancing the process of phagocytosis by interaction with the complement receptor CR1 on phagocyte surface. The CR1-C3b interaction also promotes the clearance of immune complexes, which are carried by red blood cells and removed by phagocytes in the liver and spleen.26

Regulation of CS activation is promoted by both circulating soluble proteins and proteins bound to the cell membrane. This
mechanism is species-specific, ensures that the activation of CS at low levels does not impair the organism’s own cells, and prevents the occurrence of deposition of the complexes generated on autologous cells.

**THE MAJOR HISTOCOMPATIBILITY COMPLEX**

The human major histocompatibility complex (MHC) is composed of a set of highly polymorphic genes called human leukocyte antigen (HLA) and comprises more than 120 functional genes, of which about 20% are associated with immunity. The association between autoimmune diseases and MHC genes reflects the important role of these molecules in directing the immune response. For its role in antigen presentation, MHC provides a link between innate response and adaptive response. In humans, these genes are located on chromosome 6 and are traditionally divided into classes I, II, and III. Only the genes of classes I and II are involved in presenting antigen protein to LT. Class I molecules are present on the surface of all nucleated cells, while class II are found primarily on APCs (macrophages, DCs, and LB). All MHC molecules found in the surface of a cell have an associated peptide. Although the molecules of classes I and II exhibit different structural characteristics, both are expressed as heterotrimers in which two chains are from MHC molecule and the third is the peptide presented to TL. Class I molecules are present on the surface of all nucleated cells, while class II are found primarily on APCs (macrophages, DCs, and LB).

**Figure 6**

Relative genomic position of genes within the HLA region of chromosome 6 short arm, which contains the human MHC Class I (A) and II (B). Peptide chains molecules of MHC class I and class II (S). Roadmap for the nomenclature interpretation of specificity and alleles of the major histocompatibility complex - MHC (D).

HLA class II molecules consist of two chains, α and β, both encoded by polymorphic genes in MHC class II complex regions (Figure 6 B). The α and β chains of class II molecules are encoded by genes HLA-DR, DP, and DQ families. Typically, an α chain of any type (for example, DR type) is associated with a β chain of the same type, but there may be heterologous pairing; thus, depending on the degree of homozygosity or heterozygosity, an individual may develop on the surface of his APCs 10 to 20 different class II molecules. In the nomenclature of genes in class II, the first letter indicates the class (D), the second indicates the family (M, O, P, Q, R) and the third indicates the chain A (α) or B (β). Individual genes of each of these families are distinguished by numbers, and complete nomenclature of an allelic variant is preceded by an asterisk. For example, HLA-DRB1*0101 means the 0101 allele of gene 1 encoding the β chain of class II molecule of the DR family (Figure 6 D). The HLA class II molecules present exogenous peptides to TL, i.e., derived from proteolysis of non-autologous proteins in phagolysosomes.

**INNATE IMMUNITY IN THE CONTEXT OF INFLAMMATORY RESPONSE**

The first line of defense of the organism to tissue damage is an inflammatory response, a complex biological process that involves vascular and cellular components and a variety of soluble substances, besides having characteristic clinical signs, such as redness, warmth, swelling, pain, and functional
impairment. The purpose of this process is to remove the stimulus inducing the response and start local tissue recovery. When inflammation is taking place, several biochemical systems, such as CS and coagulation cascades, are activated to help the establishment, development, and resolution of the process. Additionally, short half-life soluble substances are released, which exert their action and are degraded. Typically, the successful removal of the triggering stimulus leads to the resolution of acute response and complete tissue repair.

The acute inflammatory response evolves from a phase started by vascular cells in the tissue immediately after the injury. At baseline, only a fraction of the capillaries comprising the tissue is permeable, but after injury, local vasodilation and increased capillary permeability occur mediated by vasoactive amines, histamine, and serotonin released from mast cells and monocytes minutes after the injury. Initially, electrolytes and small molecules leave the capillary bed, forming the transudate. Subsequently, larger molecules, such as albumin and fibrinogen, also leave the capillary bed, forming the exudate. Protein output to extravascular space is accompanied by water loss and marginalization of leukocytes, which start circulating by the endothelium. The local endothelium becomes activated, expressing surface molecules that promote adherence of leukocytes and their eventual migration to the tissues. Some components of CS, kinins generating system, and coagulation system also leave to the extravascular space and are activated. Macrophages in the injured tissue release inflammatory cytokines, such as IL-1, TNF-α, and chemokines.

Migration of circulating cells into tissues, called diapedesis, is directed by the presence of a gradient of chemotactic substances in the inflammatory site. When these cells are present in tissue, they seek the phagocytosis of pathogen, allowing the tissue repair (Figure 7). In acute inflammation, the predominant elements of the innate immune response and the principal cells involved are neutrophils and macrophages. In chronic inflammation, usually caused by persistent noxious stimulation, the inflammatory process is maintained and undergoes qualitative changes, characterized by progressive change in soluble and cellular elements that infiltrate the tissue. Persistence of harmful agent leads to the chronicity of the process, with concomitant destruction and tissue repair. In chronic inflammation, tissue characteristically presents an infiltrate composed mainly of mononuclear cells (monocytes, macrophages, and lymphocytes), signs of angiogenesis, and fibrosis (Table 2). Several stimuli can induce persistent chronic inflammation, such as intracellular bacteria (e.g., Mycobacterium tuberculosis), chemical substances such as silica, and even physical agents such as ultraviolet radiation, and repetitive trauma. The mechanisms involved in chronic systemic inflammation of unknown etiology, such as rheumatoid arthritis, are not as well understood as those associated with infectious processes.

**LEUKOCYTE MIGRATION: ADHESION MOLECULES**

In normal blood flow conditions, the cells circulate in the center of the vessels, where the resistance is lower and flow rates are higher. When there is vasodilation, the rate of blood flow decreases and the circulating cells collide more often with

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**Figure 7**
Mechanisms of leukocyte migration to inflammatory site. Macrophages stimulated by inducers of inflammatory response produce cytokines, such as TNF-α and IL-1, which induce the endothelial venules to express selectins, integrins, and chemokines ligands. Selectins mediate the weak adhesion of neutrophils; integrins promote strong adhesion; and chemokines activate and stimulate the migration of neutrophils to inflammatory focus. Monocytes and activated T lymphocytes use the same mechanisms to migrate to infection sites.
activated endothelial cells, which express surface molecules capable of binding to leukocytes. The activated endothelial cells express high levels of adhesion molecules of the selectin family, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1). Endothelial activation is caused by microbial products, cytokines (IL-1, TNF-α), CS activated components, coagulation factors, histamine, and leukotriene B4. Selectins are glycoproteins present in leukocytes (L-selectin), endothelium (E-selectin and P-selectin), and platelets (P-selectin) that bind glycosylated molecules on other cells surface and, typically, mediate adhesion of low affinity between leukocytes and endothelium. Despite the low affinity, this interaction is sufficient to attract leukocytes to the periphery and promote contact with the endothelium.

Taking a neutrophil as an example, its first contact with the activated endothelium is mediated by interaction of selectins P and E on endothelium with mucin present on its surface. Concomitantly, L-selectin constitutively expressed on neutrophils binds to the set of mucin on endothelium surface. These are rapid dissociation bindings, which causes neutrophils to roll in the vessel wall driven by blood flow and being exposed to chemotactic factors. Among the chemotactic factors, we highlight the fragments of fibrin; collagen; platelet soluble factors; mediators from mast cells C5a, C3a, and C4a; residues of bacterial metabolism, such as N-formylated peptides; and chemokines secreted by different cell types. Chemokines induce changes in another set of adhesins on leukocyte surface, integrins, leading to recognition of higher avidity to ligands expressed on endothelial cells, immobilizing neutrophils and promoting their adherence to the vessel wall. The migration of cells attached to the adjacent tissue is driven by the increasing gradient of chemotactic products, facilitated by the interaction of integrins with extracellular matrix components, such as fibrin and fibronectin. Leukocyte extravasation and migration depend on the chemokines IL-8 and MCP-1, which are produced at infection sites and bind to proteoglycans on extracellular matrix and similar molecules on endothelial cells surface. The IL-8, released by activated macrophages, attracts neutrophils that are stimulated to penetrate the inflamed tissue, whereas MCP-1 recruits monocytes, T cells, NK cells, and dendritic cells later. In Figure 7, some adhesion molecules and their ligands are outlined. Dynamics of adhesion molecules dynamics varies from minutes to hours. Some molecules, such as P selectin, are found in the membrane of intracytoplasmic secretory vesicles (Weibel-Palade bodies), which rapidly fuse with plasma membrane when the cell is stimulated. Others, such as E selectin, ICAM-1, and VCAM-1 require hours for their synthesis.

### TABLE 2

**Characteristics of acute and chronic inflammatory processes**

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<thead>
<tr>
<th>Inflammation</th>
<th>Acute</th>
<th>Chronic</th>
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<tbody>
<tr>
<td><strong>Causal agent</strong></td>
<td>Organic pathogens, ionizing radiation, chemical agents, mechanical trauma</td>
<td>Initial inflammatory stimulus persistency, autoimmunity</td>
</tr>
<tr>
<td><strong>Cells involved</strong></td>
<td>Neutrophils, monocytes, macrophages, mast cells</td>
<td>Macrophages, lymphocytes, fibroblasts</td>
</tr>
<tr>
<td><strong>Primary mediators</strong></td>
<td>Vasoactive amines, eicosanoids, chemokines, reactive oxygen species</td>
<td>IFN-γ, cytokines, growth factors, hydrolytic enzymes</td>
</tr>
<tr>
<td><strong>Onset</strong></td>
<td>Immediate</td>
<td>Late</td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td>Few days</td>
<td>Months or years</td>
</tr>
<tr>
<td><strong>Evolution</strong></td>
<td>Healing with ad integrum restitution, abscess formation or chronification</td>
<td>Tissue destruction and fibrosis</td>
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**SOLUBLE MEDIATORS OF INFLAMMATORY RESPONSE**

The mediators of inflammatory response are varied and derive from precursor cell and plasma, which can be classified according to their biochemical properties in: vasoactive amines, vasoactive peptides, cleavage of CS lipid mediator products, cytokines, chemokines, and proteolytic enzymes (Tables 3 and 4). Histamine exerts its physiological effects through interaction with four different target cell receptors, H1, H2, H3, and H4. H1 promotes smooth muscle contractions of various organs and increase venous capillary permeability (drug generically known as anti-histamine receptor blockers). H2 increases secretion of gastric acid and promotes smooth muscle relaxation. H3 is involved in negative feedback synthesis of histamine H4 and mediates mast cell chemotaxis.

Bradykinin is part of the peptide family, generated in plasma by the action of enzymes on kininogens. Bradykinin B2 receptors are constitutive and mediate the increased blood flow and vascular permeability, bronchoconstriction, and algetic receptor stimulation. B1 receptors, little expressed in most tissues under normal conditions, are rapidly induced in pathological conditions by various proinflammatory stimuli, such as IL-1, IFN-γ, and TNF-α. Another group of important molecules in inflammatory process are the neuropeptides,
substance P, neurokinin A, vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP), somatostatin, and enkephalins. Substance P and CGRP have proinflammatory effects and are responsible for neurogenic inflammation. Substance P and neurokinins act on NK1 receptors, increasing blood flow and vascular permeability, and on NK2 receptors, inducing bronchoconstriction.33

Lipid mediators derived from arachidonic acid are produced by activation of phospholipases, which cleave phospholipids constituents of cell membrane generating prostaglandins, leukotrienes, and platelet-activating factor (PAF). Prostaglandins have functions as inflammatory fever, hyperalgesia, and vasodilatation, increasing edema and contraction or relaxation of smooth muscle. These mediators also participate in physiological processes, such as maintaining mucosal epithelium integrity, renal function, reproduction (fetus survival, egg implantation, uterus contraction during delivery), proliferation, and cell death.34

Inflammation provides essential signals for TL and LB activation, thus initiating the specific immune response and contributing to the integration of innate and acquired immunity.

CHEMOKINES

Chemokines are a large family of homologous cytokines structurally responsible for the movement of leukocytes, including their migration from the blood to sites of tissue

Table 4
Soluble mediators of cell derived inflammations

<table>
<thead>
<tr>
<th>Cell mediators</th>
<th>Type</th>
<th>Main source</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>Vasoactive amine</td>
<td>Mast cells, basophils, platelets</td>
<td>Present in preformed granules. Causes arterioles dilation and increased vascular permeability.</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Soluble gas</td>
<td>Macrophages, endothelial cells</td>
<td>Patent vasodilator, relaxes smooth muscle, reduces platelet aggregation, has antimicrobial activity at high concentrations.</td>
</tr>
<tr>
<td>Leukotriene B4</td>
<td>Eicosanoid derived from arachidonic acid by action of lipoxigenase</td>
<td>Leukocytes</td>
<td>Promotes leukocyte activation and adhesion to endothelium and its migration. Induces formation of reactive oxygen species in neutrophils.</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>Eicosanoid derived from arachidonic acid by action of cyclooxygenase</td>
<td>Mast Cells and Basophils</td>
<td>Cause vasodilation, fever and pain.</td>
</tr>
<tr>
<td>TNF-α and IL-1</td>
<td>Cytokines</td>
<td>Macrophages</td>
<td>Activate fibroblasts and promote leukocyte and chemotaxis adhesion. Cause systemic effects, including fever, loss of appetite and increased heart rate.</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Cytokines</td>
<td>T and NK cells</td>
<td>Antiviral, immunoregulatory and antitumoral. Also called macrophage-activating factor, is important in chronic inflammation.</td>
</tr>
<tr>
<td>IL-8</td>
<td>Chemokine</td>
<td>Macrophages</td>
<td>Neutrophil activation and chemotaxis.</td>
</tr>
</tbody>
</table>
inflammation. They are small polypeptides of 8 to 12kDa with two internal disulfide bonds. About 50 different chemokines have been identified and classified into families by the number and location of N-terminal cysteine residues. The two main families are CC chemokines with adjacent cysteine residues and CXC family, such as IL-8, where these residues are separated by one amino acid.

Chemokines can be constitutive or induced. Constitutive chemokines are normally produced in various tissues and recruit leukocytes, mainly lymphocytes, in the absence of inflammation. Induced chemokines (or inflammatory) are produced by various cells in response to inflammatory stimuli and recruit leukocytes to inflammation sites.

Chemokine receptors have seven transmembrane domains coupled to G proteins, which are present on the cell surface. It has been identified 11 different receptors for CC chemokines (CCR1 to CCR11) and seven for CXC chemokines (CXCR1 to CXCR7). These receptors may be specific to a given chemokine (e.g., CCR6, CCR9, and CXCR6), but commonly a single receptor can bind several chemokines of the same group.

Chemokines play a crucial role in mononuclear cells movement through the body and in their migration to tissues, contributing to the adaptive immune response and/or pathogenesis of many diseases. Chemokine receptors are expressed on leukocytes, dendritic cells, and Langerhans cells. The greatest variety of receptors is observed in TL and its expression can define the migratory pattern and even facilitate the identification of certain subtypes of TL. The chemokine-receptor binding initiates a complex signaling cascade that generates chemotactic responses, degranulation, release of ROS, and alteration in the affinity of integrins present on cell surface.

In addition to chemotactic agents for leukocytes, chemokines and their receptors play other important roles. Some receptors, including CCR5, are the main coreceptors for certain strains of human immunodeficiency virus (HIV). The deletion of 32 nucleotides at polymorphic variant CCR5Δ32 makes their carriers resistant to HIV infection.

Some chemokines are involved in angiogenesis by its chemotactic effect on endothelial cells, while others exert antiangiogenic effect. It is believed that chemokines also play an important role in hematopoiesis, tumor cell growth, and development of metastases. Chemokines and their receptors have also been implicated in the pathogenesis of several neurological diseases, including multiple sclerosis. Elevated levels of IL-8 have been reported in synovial tissue and synovial fluid in cases of rheumatoid arthritis and various systemic inflammatory conditions.

### INFLAMMATORY RESPONSE CLASSIFICATION

The inflammatory response is generally beneficial to the body, resulting in elimination of microorganism by phagocytosis or lysis by CS, dilution or neutralization of toxic or irritants irritating substances by local leakage of fluid rich in proteins, and limitation of initial injury by fibrin deposition. In some situations, however, it may have undesirable consequences; for example, in allergic reactions and autoimmune diseases. The exacerbated inflammatory responses mediated by the immune system, called hypersensitivity reactions, are classified according to the triggering mechanism (Table 5).

The immediate hypersensitivity reactions (type I) are characterized by the presence of IgE and, generally, triggered by an external antigen (allergen). They may be systemic, involving multiple organs, or more restrictive as in urticaria and allergic rhinitis. The interaction between allergen and IgE preformed and prefixed to surface receptors of mast cells and basophils results in the release of soluble mediators (histamines) and synthesis of lipid mediators derived from arachidonic acid. Allergic rhinitis, asthma, and anaphylactic reactions are examples of type I reactions.

Type II reactions depend on the production of antibodies (IgG and IgM classes) against a specific antigen. The fact that humoral response causes damage instead of protection depends on the nature of the antigen, immunoglobulina isotype formed, and especially the specificity and avidity of the autoantibodies in question. The damage mechanisms associated with type II reactions include lysis of cells with antigen on their surface by activation of CS; destruction by NK cells, which have Fc receptors for IgG and perform antibody-mediated cytolysis; and limitation of initial injury by fibrin deposition. In some situations, however, it may have undesirable consequences; for example, in allergic reactions and autoimmune diseases.

### Table 5

Classification of hypersensitivity reactions according to Gell and Coombs

<table>
<thead>
<tr>
<th>Type</th>
<th>Alternative name</th>
<th>Associated diseases</th>
<th>Mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Immediate</td>
<td>Asthma</td>
<td>IgE</td>
</tr>
<tr>
<td></td>
<td>hypersensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antibody-mediated hypersensitivity</td>
<td></td>
<td>IgG or IgM and complement</td>
</tr>
<tr>
<td></td>
<td>Serum sickness</td>
<td>Arthus’ Reaction</td>
<td>IgG and complement</td>
</tr>
<tr>
<td></td>
<td>Lupus nephritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Delayed</td>
<td>Transplant rejection</td>
<td>T cells, macrophages, histiocytes</td>
</tr>
<tr>
<td></td>
<td>hypersensitivity</td>
<td>Contact dermatitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tuberculosis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
and release of lytic enzymes and cytokines by neutrophils and macrophages activated by binding of Fc receptors to IgG.

Type III reactions are caused by the formation of antigen-antibody immune complexes (IC), which are deposited in tissues and activate CS. The only antibodies involved are the ones capable of activating complement, IgM, IgA, and all IgG subclasses, except IgG4.

Circulating IC is able to deposit in blood vessels, basement membrane of glomeruli and joints; and CS activation leads to tissue inflammation, which can result in rash, erythema nodosum, vasculitis, nephritis, arthritis, and pneumonitis. This type hypersensitivity reaction is found in various autoimmune diseases, such as lupus erythematosus, different types of vasculitis, and severe forms of rheumatoid arthritis.

Type IV reactions, or delayed type hypersensitivity, are mediated by TLs, macrophages, histiocytes, and monocytes. Cytotoxic T lymphocytes (CD8) cause direct tissue damage, mediated by TLs, macrophages, histiocytes, and monocytes. Macrophages are responsible for the magnitude of tissue injury and granuloma formation characteristic of the infectious agent or foreign body persistence. Classic examples of type IV reaction are tuberculosis and leprosy in its tuberculoid form. Giant cell vasculitis and Takayasu’s arteritis also seem to arise from mechanisms related to type IV hypersensitivity.

**REFERENCES**