1 The Immune System

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1.1 Definition

The immune system is a network of cells and proteins that interact in tissues and organs to protect the body from infection, and also to promote healing. In general, immunity involves: the generation of inflammation; the removal and destruction of pathogens; the expansion of immune cell population and development of memory, specifically against the antigenic insult; control of inflammation; and tissue repair. The immune mechanisms aim to recognize and attack non-self molecules, although dysregulation can cause immunodeficiencies (e.g., insufficient protective response) or immune-mediated damage of self-molecules (i.e., autoimmune diseases and hypersensitivity reactions).

Classically defined, the immune system promotes immunity through *innate* and *adapted* segments. The innate immune cells and proteins provide immediate response and action against pathogens in a somewhat unspecific manner, while the adapted immune cells require priming with pathogen, cell co-stimulation and activation before function, and development of memory.

Immune cells cross-activate or cross-repress each other through cell-cell interactions, and in response to *cytokines* and *chemokines*, which are secreted proteins that function primarily in autocrine and paracrine manners and, sometimes, endocrine. Ligand-receptor or cytokine-cytokine receptor interactions lead to corresponding cell signaling, transcription and translation for immunostimulatory or immunosuppressive outcomes.

1.2 The organs of the immune system

The organs of the immune system are referred to as *central* (e.g., bone marrow and thymus), where cells are produced and go through initial or complete development; or *peripheral* (e.g., lymph nodes, spleen, and mucosa-associated lymphoid tissues (MALT), also known as bronchus-associated (BALT) and gut-associated (GALT) lymphoid tissues), in which cells complete their development and become activated upon encountering antigen.

Epithelial cells of the skin and mucosa comprise anatomical barriers to pathogens and toxins. Secretions (e.g., lysozymes in saliva and tears, low stomach pH) and clearance mechanisms (e.g., mucociliary system of the respiratory tract) add protection as physiological barriers. In addition, epithelial and endothelial cells can become activated by pathogens and secrete cytokines (e.g., interferon-beta, TNF-alpha), chemokines and selectins/integrins, which attract inflammatory cells. Damage to these structures and mechanisms decreases protection and favors pathogen invasion and replication.

Immune cells circulate through blood and lymph throughout the body, and migrate to tissues and lymphoid tissues, often attracted by chemokines; they can either settle and become resident cells, or constantly recirculate in search of an antigen or a site of inflammation. Cells circulating in the lymphatics re-enter the blood circulation via the thoracic duct and, from the blood, they can be attracted and migrate to tissues via diapedesis. From tissues, they can reach regional lymphoid structures and draining lymph nodes via draining lymphatics. Immune cells and antigens reach the spleen via blood, which works as a filter, with small capillary structures surrounded by organized lymphoid tissues.

Diapedesis is the process of extravasation of leukocytes from the blood stream into tissues. Sentinel cells of the immune system resident at tissue sites (e.g., macrophages, mast cells) detect the presence of pathogens or tissue destruction through their receptors. Cell signaling, transcription and translation follow, and these cells secrete inflammatory cytokines (e.g., tumor necrosis factor-alpha, TNF-alpha; interleukin-1,IL-1; IL-6) and chemokines (e.g., IL-8), which attract other inflammatory cells to the site. They cause fever and vasodilation, and increase capillary permeability, responsible for the clinical signs observed during inflammation (Chapter 18, Table 18.1).

The inflammatory cytokines also induce the expression of *adhesion molecules* called selectins (e.g., E-selectin) and integrins (e.g., vascular cell adhesion molecule-1, VCAM-1) on the luminal surface of local endothelial cells that bind (initially gently, then tightly) to the surface of leukocytes in the blood flow. This process induces the expression of similar adhesion molecules on the leukocyte surfaces (e.g., L-selectin; integrin CD11a-CD18 or LFA-1 lymphocyte-function associate antigen 1). With time, leukocytes roll along the luminal endothelium, then attach tightly to the endothelial cells and, finally, pass through gaps

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between them. A similar process is used in the absence of inflammation, when monocytes leave the blood stream into tissues and become resident macrophages, the immune sentinels. Once in the tissue, leukocytes follow the chemokine (e.g., interleukin-8, IL-8; complement component C5a) gradient produced by the macrophages and other inflammatory cells and proteins, and find the site of inflammation.

1.3 The immune cells and soluble molecules

The immune cells originate from myeloid and lymphoid precursors in the bone marrow, and follow stepwise genetically and epigenetically controlled lineage differentiation from hematopoietic stem cells. The bone marrow milieu has cell lineage niches that receive and respond to systemic signals (i.e., hormones, cytokines) with the production of new hematopoietic cells.

1.3.1 Myeloid cells

Myeloid cells comprise neutrophils, monocytes, macrophages, dendritic cells, eosinophils, basophils, mast cells, red cells and thrombocytes. These cells can complete maturation in the bone marrow, although further differentiation upon antigen encounter may happen at peripheral sites (e.g., monocytes differentiating into macrophages). Neutrophils, monocytes, macrophages and dendritic cells are phagocytes. They recognize pathogens, phagocytose and kill them, becoming activated during this process, and secreting cytokines and chemokines to signal other cells and expand the inflammatory response (Chapter 18, Table 18.4).

The recognition of pathogens by phagocytes is based on their *pattern-recognition receptors* (PRR). Signaling PRR include Toll-like receptors (TLRs, e.g., TLR-2, TLR-4, TLR-7 – about 11 described thus far); nucleotide-binding oligomerization receptors (NOD-like receptors – about 20 described thus far); or retinoic-acid inducible protein-1 (RIG-1-like receptors, also known as RLRs) (Figure 1.1; see also Chapter 18, Table 18.3).

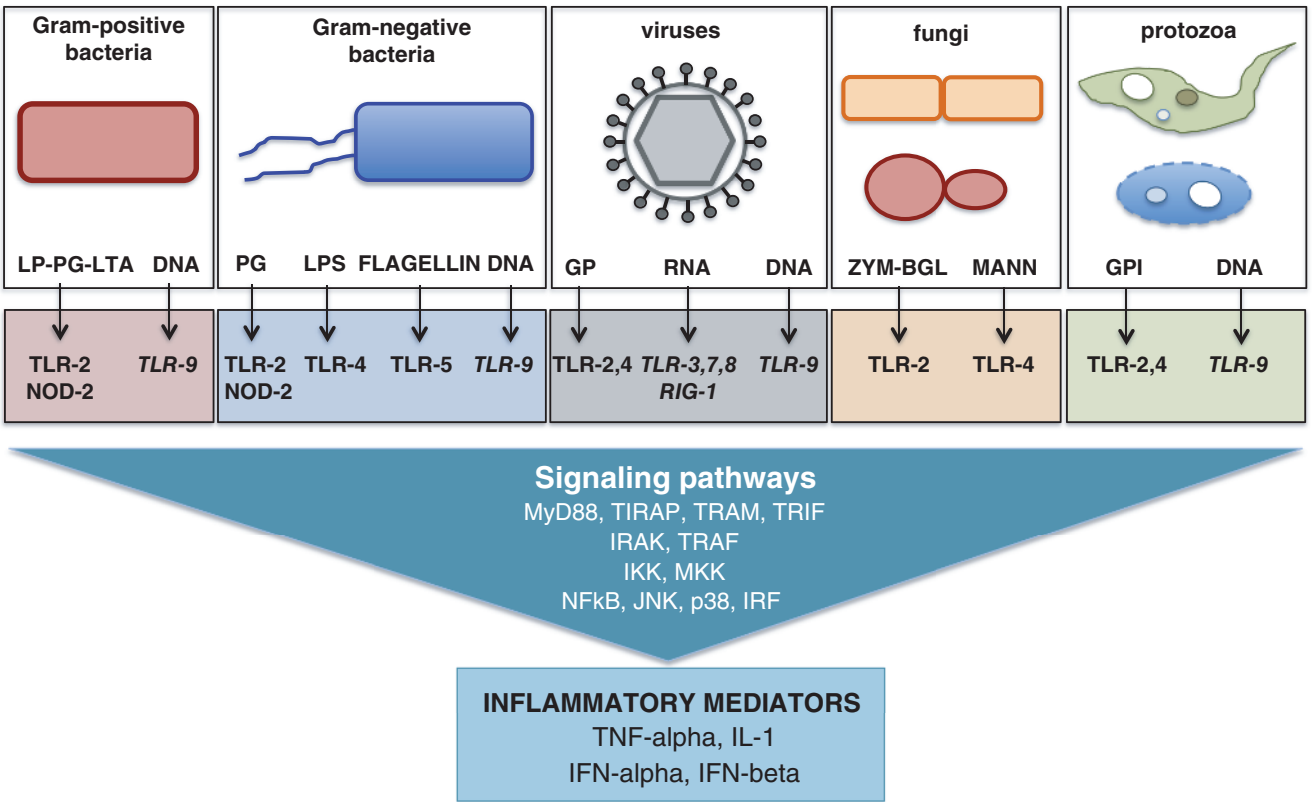

Figure 1.1 The pathogen-associated molecular patterns (PAMPs) and pathogen-pattern receptors (PRRs).
Pathogen small molecular motifs (PAMPs) or extracts (vaccines) can be detected by cell-membrane or intracellular (endosomic) receptors (PRRs) in phagocytes (e.g., macrophages and dendritic cells). PAMP-PRR binding triggers receptor-specific cell signaling events that lead to the production of inflammatory mediators (pro-inflammatory cytokines and type 1 interferons). Bacterial lipopeptide (LP), peptidoglycan (PG), lipoteichoic acid (LTA), lipopolysaccharide (LPS), and flagellin are detected by different cell-membrane toll-like receptors (TLRs) and intracellular nucleotide-binding oligomerization receptors (NOD-like receptors). Viral glycoprotein is detected by TLR-2 and TLR-4. Protozoal-released glycosylphosphatidylinositol (GPI)-anchored proteins are recognized by TLR-2 and TLR-4. Fungal zymogen and beta-glucosidase (BGL) are recognized by TLR-2, and mannose by TLR-4. Viral nucleic acids ribonucleic acid (RNA) is detected by intracellular TLR-3, TLR-7 and TLR-8, and retinoic-acid inducible protein-1 (RIG-1-like receptors); bacterial, viral and protozoal DNA (i.e., unmethylated cytosine-phosphodiester-guanine deoxynucleotide (CpG) motif) is recognized by intracellular TLR-9.

Located on the cell membrane or in the cytosol, they detect extracellular and intracellular pathogens, respectively. Each of these receptors recognizes distinct *pathogen-associated molecular patterns* (PAMPs), for which it has affinity.

In addition, endogenous molecules released by cell damage serve as *danger* alert, also known as *damage-associated molecular patterns* (DAMPs). As signaling receptors, PRR binding to a certain PAMP induces a determined cell-signaling configuration that results in the transcription and translation of a determined type of inflammatory response. Hence, the type of pathogen, defined by its signature molecular pattern, determines the type of immune response that, hopefully, will eliminate it. Early danger signals will recruit inflammatory cells that promptly potentiate this capacity.

Phagocytosis is the process of engulfing particles (endocytosis), including organisms, cells, proteins and other molecules. Phagocytes use different receptors to initiate phagocytosis. Some of them bind directly to the pathogens (e.g., mannose-receptors), while others require opsonization (*coating*) of the organism with immunoglobulin or complement, so they can bind to immunoglobulin or complement receptors on phagocytes. Once the pathogen adheres to the phagocyte surface, the cellular actin-myosin system contracts and expands the cell to engulf the pathogen and form a phagosome, which is rapidly fused with a cytosolic lysosome to form a *phagolysosome*. In the phagolysosome, proteolytic enzymes, oxygen-reactive species (e.g., hydrogen peroxide, hypochlorite, superoxide) and nitrogen-reactive species (e.g., peroxynitrite) produced through oxidative burst activity degrade the pathogens, and waste material is expelled from the cell.

Neutrophils are short-lived cells that arrive very quickly at the site of inflammation and competently phagocytose and kill pathogens, along with macrophages. Phagocytosis is much efficient when the pathogen is opsonized, creating a certain dependence on immunoglobulins and complement for phagocytic function. In addition, for some organisms (e.g., encapsulated bacteria), phagocytosis and killing require opsonization with both immunoglobulin and complement. As the early phase of inflammatory response supported primarily by neutrophils controls infection, macrophages become the predominant anti-inflammatory cells for tissue repair. Nevertheless, persistence of infection leads to chronic inflammation, with imbalanced tissue damage and repair.

Macrophages originate from circulating monocytes but live in tissues. They receive different names, accordingly: histiocytes, microglia, alveolar macrophages, osteoclasts, giant cells. There are pro-inflammatory macrophages (M1 cells) that are involved in the inflammatory response described above, and anti-inflammatory macrophages (M2 cells), which are involved in tissue repair. These cells co-exist during inflammation but their roles are more predominant in one phase or another, and both cell types have important roles. These cells require phenotypic and functional analyses for characterization. For example, exposure to the pro-inflammatory cytokine interferon-gamma (IFN-gamma) and PAMP lipopolysaccharide (LPS, endotoxin) activate TLR-4 and differentiate macrophages into M1 cells, which subsequently express more pro-inflammatory cytokines (e.g., IL-12, IL-23, TNF-alpha), and promote inflammatory responses by activating lymphocytes and neutrophils. In addition, activation of M1 cells leads to the production of enzymes involved in oxidative burst activity, increasing the risk of tissue damage via oxidative stress. M2 cells, on the other hand, differentiate from exposure to IL-4, and secrete the anti-inflammatory cytokine IL-10. They promote tissue remodeling and decrease inflammation, which can also favor the development of tumors.

Dendritic cells and B cells can also function as phagocytes with lower efficiency for pathogen removal and killing, but with the objective to interact with T lymphocytes. Dendritic cells and Langerhans cells (skin) have the same PRRs for pathogen recognition and mechanisms for phagocytosis. Upon pathogen binding, immature dendritic cells found in tissues become mature cells and migrate to the regional lymph nodes, where they function as antigen presenting cells (APCs). Dendritic cells process killed extracellular pathogens into small peptides that are loaded to molecules called major histocompatibility (MHC) class II. The expression of MHC class II is increased after maturation, and the dendritic cells use their large cell surface area (proportionate to their dendritis) to express high amounts of MHC molecules loaded with foreign peptides.

The foreign peptides presented with MHC class II molecules may be recognized by a $CD4^+$ T cell that has a T cell receptor (TCR) with affinity for the peptides, and become partially activated (Chapter 13, Figure 13.1). Dendritic cells then express co-stimulatory molecules (CD40, CD86), which reinforce T cell activation when they bind to ligands on $CD4^+$ T cells (CD40L, CD28, respectively). In addition, dendritic cells secrete cytokines (e.g., IL-12) that can modulate and activate $CD4^+$ T cells and induce their proliferation. In essence, dendritic cells promote the development and guide the direction of the acquired immunity, based on the nature of its own antigenic stimulus (i.e., the PRR and cell signaling induced by the PAMP encountered). Macrophages are also capable of antigen presentation but perhaps dendritic cells provide greater co-stimulatory support to naïve T cells during their first antigen encounter.

Cell markers used for myeloid cell recognition in immunologic testing for horses include CD11b, CD14, CD172a, CD86, CD250, and MHC class II. Monocytes, macrophages and dendritic cells express these markers in different quantities, according to their stage of development and activation.

Eosinophils, basophils (circulating) and mast cells (tissue residents) are granular cells that participate in cytotoxicity reactions against parasites and allergens. Eosinophils respond to IL-5 for activation and proliferation. Basophils and mast cells express epsilon receptors for IgE, and most IgE is bound to these receptors by its constant region; upon allergen/antigen binding to the variable region of IgE, immediate cell degranulation releases vasoactive amines (e.g., histamine, leukotrienes, heparin, proteolytic enzymes), which cause cytotoxicity, vascular

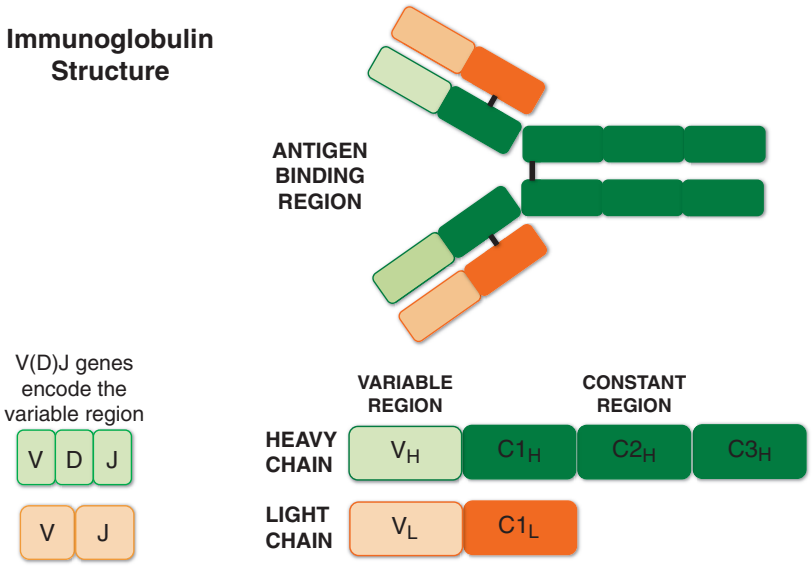

Figure 1.2 The immunoglobulin structure. Immunoglobulin has two heavy chains (with variable and constant regions) and two light chains (also with variable and shorter constant regions) that form a 'Y' shaped structure. The heavy and light chain variable regions bind to antigens; and the heavy chain constant region determines the immunoglobulin isotype (IgA, IgE, IgG, or IgM) and promotes specific immune functions. The diversity in the variable region of the immunoglobulin is achieved by DNA recombination during B cell development, precisely of a region of genes named V (variability), D (diversity) and J (joining).

permeability, and the release of cytokines (an important source of IL-4) (see Chapter 4, Figure 4.1 and Chapter 8, Figure 8.2).

Myeloid cells play an important role in the detection and removal of blood-borne pathogens and particles, and aged erythrocytes and platelets, through the mononuclear phagocyte system and the pulmonary intravascular macrophages. The mononuclear phagocyte system (formerly known as the reticuloendothelial system) comprises monocyte precursors in the bone marrow, circulating monocytes and tissue macrophages. Pulmonary intravascular macrophages are resident cells of the capillary endothelium of lungs, and have a similar morphology to the Kupfer cells of the liver. Together, these cells have the phagocytic, microbicidal, and secretory properties of other phagocytes. It is possible that the detection of and response to immunomodulators (e.g., bacterial extracts, endotoxin) is initiated by the pulmonary intravascular macrophages in the horse.

1.3.2 Lymphoid cells

Lymphoid cells derive from common lymphoid progenitors in the bone marrow and comprise B and T lymphocytes, and natural killer (NK) cells.

B lymphocytes complete their initial development into immature B cells in the bone marrow in the absence of foreign antigens and, subsequently, B cells continue their differentiation and selection after antigen encounter in peripheral tissues. The goal of a B cell during development in the bone marrow is to form a B cell receptor (BCR) that does not recognize self-molecules, but can individually and specifically recognize an infinite number of structures (molecules) present in the vast world of pathogens (antigens). Consequently, a very large number of autoreactive cells or cells that cannot build their BCR are eliminated daily during this process. The BCR is formed by a cell-surface bound IgM molecule and supporting transmembrane molecules (CD19, CD79a, CD79b), which promote cell signaling and activation of B cells upon antigen binding to the receptor.

The immunoglobulin structure has two heavy chains (with variable and constant regions) and two light chains (also with variable and shorter constant regions) that form a 'Y' shaped structure. The heavy and light chain variable regions (forming the 'V') bind to antigens; and the heavy chain constant region (the 'I') determines the immunoglobulin isotype and promotes specific immune functions (Figure 1.2).

When B cells are developed in the bone marrow, each cell is produced with different variable regions in order to create an arsenal of cells that recognizes the enormous variety of antigens/pathogens to which an individual is exposed in a lifetime. This diversity is achieved thanks to DNA recombination during B cell development, precisely of a region of genes named V (variability), D (diversity) and J (joining). Different gene segments from these regions are trimmed and reunited (recombination) to form diverse exons that, consequently, encode different variable regions of the heavy or light chains of the immunoglobulin. Diversity is additionally obtained by the addition of nucleotides during the recombination process that reunite the DNA segments (junctional diversity, using N and P nucleotides). Diversity also follows in the periphery, with point mutations in the variable region to improve antigen recognition and binding. The constant region of a developing B cell is of IgM isotype. Once a B cell accomplishes its BCR on the cell surface and tests negative for self-recognition, it leaves the bone marrow towards peripheral tissues, in search of its matching foreign antigen. Cell markers used for B cell recognition in horses include B220, CD19, CD20, CD21, CD79a, IgG, and IgM.

T lymphocytes are long-lived cells that originate in the bone marrow from a common lymphoid progenitor, and move to the thymus to complete their differentiation; they also produce a T cell receptor. T cell receptors are heterodimers formed by alpha and beta chains (alpha-beta T cell), or gamma and delta chains

(gamma-delta T cells), each with variable and constant regions. The variable regions are put together using the same process for the immunoglobulins in B cells. Alpha and beta TCRs need to be positively selected for their fit to the host MHC class I or class II molecules; if a TCR fails to recognize a MHC molecule, it is eliminated. An alpha-beta T cell that recognizes MHC class I becomes a $CD8^+$ (cytotoxic) T cell (CTL); an alpha-beta T cell that recognizes MHC class II becomes a $CD4^+$ (helper) T cell. A CD3 molecule for transmembrane signaling accompanies alpha-beta TCRs. T cells that recognize self-peptides (autoreactive) are eliminated in the thymus and are not released in the circulation. Cell markers used for alpha-beta T cell recognition in horses include CD3, CD4, and CD8.

Gamma-delta T cells also develop in the thymus and have diverse TCR, but are not restricted to recognition of peptides bound to MHC molecules (i.e., they can also bind to whole molecules), and they perhaps offer complementary mechanisms to detect antigen in the early stages of infection, likewise innate immune cells. They circulate in blood and tissues, and express a variety of cytokines (IFN-gamma, TNF-alpha, IL-17, IL-4) that signal inflammation upon activation. Gamma-delta T cells have not been thoroughly described in the horse and, if present, they represent a small population.

Regulatory $CD4^+$ T cells (Tregs) can develop in the thymus or in the periphery (induced Tregs), and comprise a small percentage of the circulating T cells. Tregs are heterogeneous cell populations that suppress the activation and proliferation of effector T cells; they play an important role in preventing tissue damage and autoimmunity, and provide peripheral immunotolerance. Their suppressive mechanism is not completely known, but involves the anti-inflammatory cytokines IL-10 and TGF-beta. Subpopulations of Tregs have been described in the horse. Cell markers used for Tregs include CD4, CD25, FoxP3, and IL-10; however, these markers are not exclusive to Tregs, and are also present in $CD4^+$ effector T cells.

The MHC class I and MHC class II molecules are polymorphic, and are encoded by different genes among individuals; these are the molecules evaluated before transplantation procedures for tissue compatibility, as they can function as foreign antigens in the recipient, and induce immune response and rejection. MHC class II is expressed primarily in APCs, but is also constitutively expressed in equine lymphocytes, and conditionally expressed in some activated cells (e.g., endothelial cells).

MHC class II molecules are loaded with processed peptides (between 18 and 20 amino acids) after phagocytosis and killing of extracellular organisms (Figure 1.3). The peptide presented in the context of the MHC class II molecule can be recognized by a TCR on $CD4^+$ T cells with specific affinity for it (Figure 1.4). MCH class I molecules are expressed in almost all nucleated cells of the body; they are also polymorphic, and are implicated in transplantation. MHC class I molecules are loaded with endogenously processed self- or non-self (e.g., tumoral, viral) peptides (between 8 and 10 amino acids) (Figure 1.3). The peptide presented in the context of the MHC class I molecule can be recognized by a TCR on $CD8^+$ T cells with specific affinity for it (Figure 1.4).

The $CD8^+$ (cytotoxic) T cells (CTLs) are activated by antigen presentation via MHC class I on APCs (cross-presentation), the co-stimulation from molecules expressed on activated $CD4^+$ T cells, and their secreted IFN-gamma. In the periphery, activated CTLs search for infected cells that express processed pathogen peptides in the context of MHC class I molecules. $CD8^+$ T cells kill infected target cells using elements that alter cell permeability (e.g., perforins, granzymes).

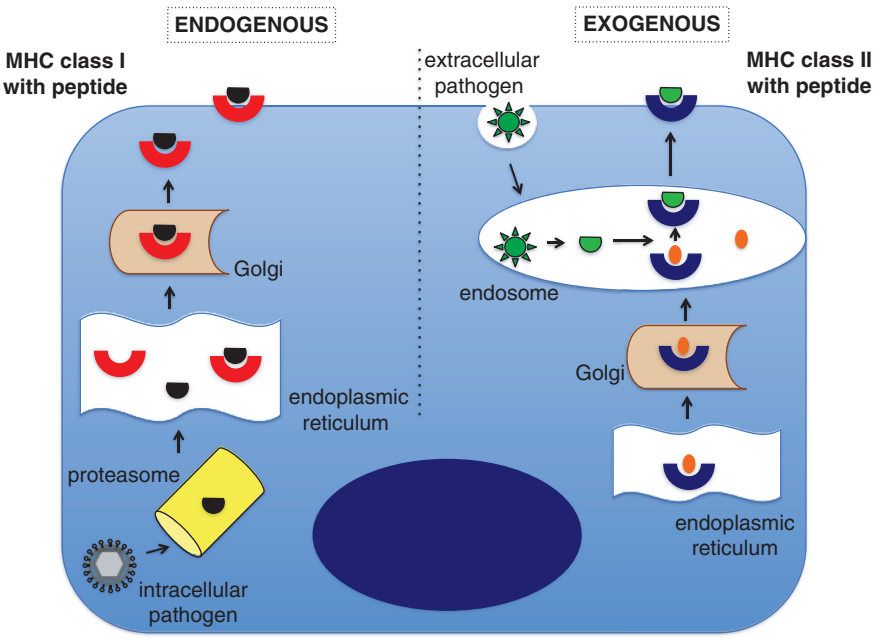

Figure 1.3 Pathways for peptide processing and loading in major histocompatibility complex (MHC) molecules.
(*left*): Endogenous pathway for peptide processing. An intracellular pathogen (e.g., virus) is processed through the proteasome and broken into small peptides (8–10 amino acids), which are loaded into a MHC class I molecule in the endoplasmic reticulum. The complex is moved through the Golgi apparatus and expressed on the cell surface. Some viruses and tumoral cells can interfere in many points of this pathway to prevent antigen presentation to CD8 cytotoxic T cells (CTLs).
(*right*): Exogenous pathway for peptide processing. An extracellular pathogen is endocytosed and broken by lysozymes into small peptides (18–20 amino acids), which are loaded in the MHC class II molecule in the endosome and expressed on the cell surface for presentation to CD4 T cells.

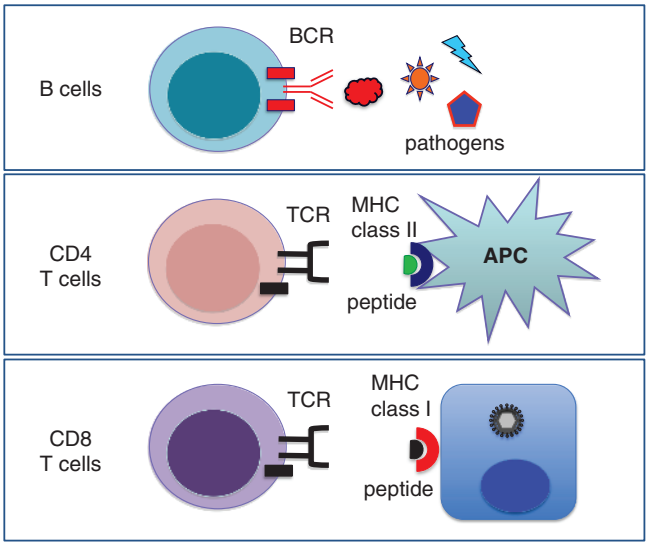

Figure 1.4 Antigen recognition by lymphocytes.
B and T cells recognize and bind specifically to antigens through their receptors, B cell receptor (BCR) and T cell receptor (TCR), respectively. The variable region of the BCR directly binds *unprocessed* antigen. On the other hand, the variable region of the TCR binds to *processed*, small peptides presented in the context of major histocompatibility (MHC) molecules. CD4 T cells recognize peptides presented by MHC class II molecules, and CD8 T cells recognize peptides presented by MHC class I molecules. Binding to antigen is the first signal for activation, and co-stimulation (with cytokines and interaction of co-stimulatory cell surface molecules) is required for full activation.

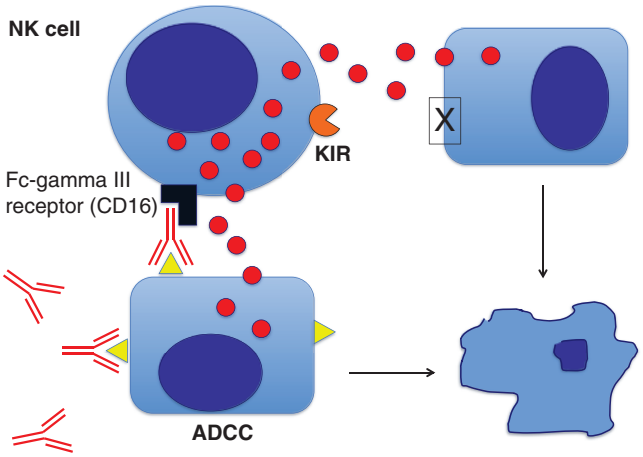

Figure 1.5 Natural killer (NK) cells kill infected cells.
NK cells recognize infected cells when they do not express MHC class I, a mechanism used by viral and tumoral cells to escape cytotoxic T cell (CTL) immunity. Without the expression of MHC class I (demonstrated by X), the target cells cannot inhibit the activation of the killer-cell immunoglobulin-like receptor (KIR). Subsequently, NK cells release granzymes and perforins (red circles) that destroy cell membranes of the target cells. In addition, an NK cell can be activated when its Fc gamma RIII receptor (CD16, its classic marker) binds IgG attached to target cells expressing non-self antigens, a cell-destruction mechanism known as antibody-dependent cell-mediated cytotoxicity (ADCC).

Natural killer (NK) cells are large granular cells, which also develop from common lymphoid precursors in the bone marrow but do not go through thymic maturation and do not express TCR or BCR. Hence, they are considered innate immune cells. NK cells kill target cells that do not express MHC class I, using granzymes and perforins that destroy cell membranes. Tumoral cells and virus-infected cells can downregulate the expression of MHC class I in order to escape $CD8^+$ T cell cytotoxicity. Without the expression of MHC class I, the target cells cannot inhibit the killer-cell immunoglobulin-like receptor (KIR), and cannot prevent the activation of NK cells (Figure 1.5). In addition, an NK cell can be activated when its Fc gamma RIII receptor (CD16, its classic marker) binds IgG attached to target cells expressing non-self antigens, a cell-destruction mechanism known as antibody-dependent cell-mediated cytotoxicity (ADCC). NK cells can become activated by lectins, and subsequently secrete inflammatory cytokines IL-2, IFN-gamma, and TNF-alpha.

1.3.3 Complement system

The complement system consists of circulating glycoproteins (C1 to C9) produced in the liver in response to inflammation. These pro-proteins are inactive until pathways induce sequential proteases that trigger the protein activation cascade:

- *classical* (antigen is bound by IgG or IgM molecules, and the C1-complex becomes activated when it binds to the antibodies);
- *lectin* (antigen is bound by mannose-binding lectin and ficolins, and C4 becomes activated); or
- *alternative* (spontaneous complement C3 activation).

The ultimate outcome is the formation of the membrane attack complex (MAC) that creates pores on the cell surface of cells, with consequent lysis. After activation, opsonic, chemoattractant, anaphylactic and cytotoxic fragments are produced. Complement glycoproteins are regulated by circulating or membrane-bound (e.g., CD59) complement control proteins, which prevent activation of early or late complement cascade events.

Phagocytic activity is facilitated by complement, which opsonizes foreign structures, and binds to complement receptors on phagocytes.

1.4 B and T cell activation in lymphoid tissues

Upon phagocytosis of antigen in tissues, dendritic cells migrate to regional lymph nodes to interact with $CD4^+$ helper T cells (Figure 1.6A). Dendritic cells present processed antigen peptide via MHC class II that binds to the TCR, and conclude $CD4^+$ helper T activation with co-stimulatory molecules CD40 (binds to CD40L in lymphocytes) and CD86 (binds to CD28 in lymphocytes), and cytokines (Figure 1.6B). This is the time that determines the fate of $CD4^+$ helper T cells. Cytokine IL-12 secreted by APCs induces Th1 cells, whereas IL-23 induces Th17 cells, and IL-4 is necessary for Th2 differentiation (although its origin is not well determined) (see Chapter 13, Figure 13.2 and

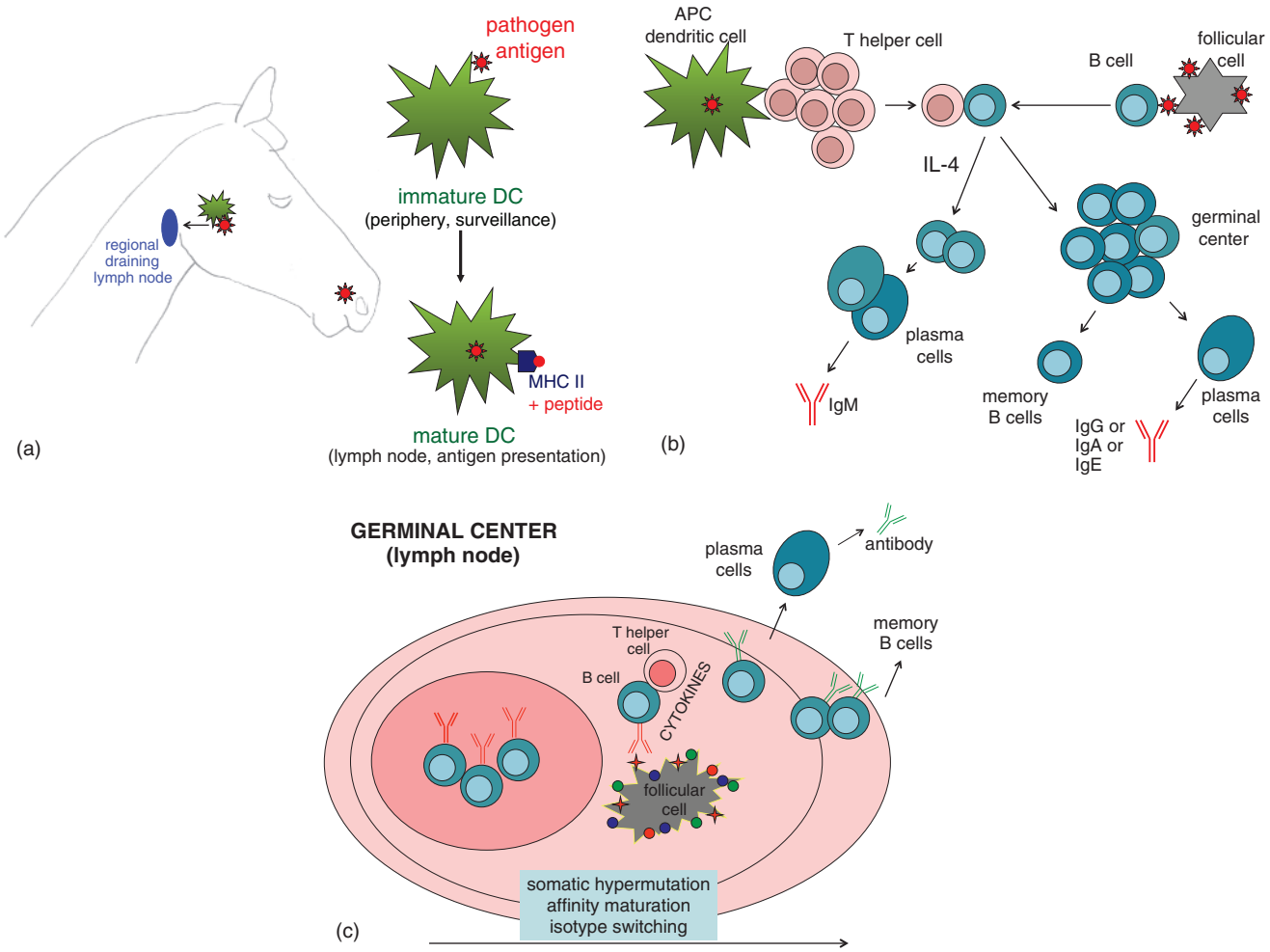

Figure 1.6 B and T cell activation in lymphoid tissues.

(a) Immature dendritic cells in peripheral tissues encounter, phagocytose and process antigen (e.g., respiratory pathogen, intranasal vaccine) and migrate to regional (draining) lymph nodes, where they become mature.

(b) In the paracortical region, they interact with naïve CD4 T cells, which become activated (helper cells) and proliferate. Also in the paracortical region, naïve IgM^+ B cells encounter their matching unprocessed antigen on the surface of follicular cells, which initiates cell activation. Nevertheless, B cells require interaction with CD4 helper T cells in order to get second signals for full activation and, subsequent, proliferation. Some of the proliferating B cells, in the presence of IL-4 secreted by Th2 cells, quickly differentiate into plasma cells and secrete IgM. The rest of the activated B cells migrate to the cortical region and form germinal centers.

(c) Germinal centers contain highly proliferative B cells that undergo point mutation (somatic hypermutation) in their variable region to increase affinity and avidity for the triggering antigens. The new variable region needs to be tested again with binding to antigen (provided by follicular cells) and another round of interaction with CD4 T cells (affinity maturation). After approval, B cells keep their variable region, but change their constant region through isotype switching into IgA, IgE or IgG. Finally, at this point, some B cells become plasma cells (which secret antibodies) or long-lived memory cells.

Chapter 25, Figure 25.2). The many types of $CD4^+$ helper T cells express the same cell markers, and can only be defined by the cytokines they secrete. Among many other types of helper T cells, Th1 cells secrete IFN-gamma, Th2 cells secrete IL-4, IL-5, IL-9, IL-13, and Th17 cells secrete IL-17.

After immature B cells leave the bone marrow, they also circulate in lymphoid tissues to encounter antigens. Whole (unprocessed) antigens reach the lymph nodes through lymph, the spleen through the blood, and the MALT through M cells in the mucosal epithelium. Antigens are often opsonized by local complement or immunoglobulins, which allows them to bind to the surface of follicular dendritic cells. These cells are not hematopoietic, and do not process antigens, but serve as a *buffet* of antigens to B cells in search of a match for their BCRs (opsonized antigens bind to Fc receptors and complement receptors on the surface of follicular cells, exposing the antigen to B cells).

When the BCR binds to antigens, it triggers the first signal for activation of B cells. However, a second signal is necessary, and it comes from the activated $CD4^+$ helper T cells. Both B cells and $CD4^+$ T cells are activated by the same triggering antigen (organism), but BCRs (cell surface immunoglobulin) can bind to unprocessed antigen (i.e., directly to the pathogen),

whereas TCR can only bind to processed antigens (i.e., peptides presented via MHC class II molecule).

B cells interact with $CD4^+$ helper T cells in the same manner in which APCs interact with them, namely, via MHC class II molecule presenting a processed peptide. After the BCR binds to and internalizes the antigen, it is processed into small peptides, which are presented to $CD4^+$ helper T cells via MHC class II. Co-stimulation with CD40-CD40L and CD86-CD28 molecules reinforce the interaction (hence, B cells are also APCS).

Once a B cell receives both signals (antigen binding and $CD4^+$ helper T cell co-simulation), it proliferates for clonal expansion. Some of the clonal cells, in the presence of IL-4 secreted by $CD4^+$ helper T cells (Th2 cells), differentiate immediately into plasma cells and secrete IgM, which is the same immunoglobulin expressed on the cell surface during bone marrow development. IgM, therefore, is the predominant immunoglobulin produced in a primary response or immunization. Part of the proliferating B cells form germinal centers, in which further B differentiation and proliferation occurs.

In the germinal centers, rapidly proliferating B cells (also known as centrocytes) develop point mutations (somatic hypermutation) in the immunoglobulin variable region (known as the complementarity determining region) to increase affinity and avidity for the triggering antigen (Figure 1.6C). Once the immunoglobulin changes its variable region, it needs to be re-tested against the antigen (provided by follicular dendritic cells) to check for improvement or deterioration of binding. If there is improvement, B cells interact again with $CD4^+$ helper T cells to receive survival signals, and this process is called affinity maturation. At this point, no more changes happen in the variable region of the cell surface immunoglobulin (BCR), and the B cell proceeds to switch the immunoglobulin isotype from IgM into IgG, IgA or IgE. The cells then differentiate into plasma cells and secrete antibodies, or differentiate into memory cells that can be activated by antigens in a subsequent exposure. The immunoglobulin isotype is determined by the cytokine milieu created by the interacting T cells.

1.4.1 Immunoglobulins

There are five major isotypes of immunoglobulins (IgM, IgG, can, IgE and IgD). These isotypes are distinguished by their heavy chain constant regions encoded by the genes mu, gamma, alpha, epsilon and delta, respectively. Each immunoglobulin isotype promotes distinct functions for neutralization, complement fixation and opsonization, and mucosal protection (Figures 1.7A and 1.7B). The combination of isotypes provides a functional strategy for removal of pathogens, and lack of specific isotypes may facilitate certain pathogen establishment and disease. For example:

1 IgG, IgA and IgM directly neutralize bacteria, viruses and toxins, preventing them to bind to cell surface ligands for invasion or dysfunction.
2 IgG and IgA opsonize organisms for phagocytosis.
3 IgG and IgM activate complement component C1q.
4 IgG participates in ADCC.
5 IgE degranulates mast cells.

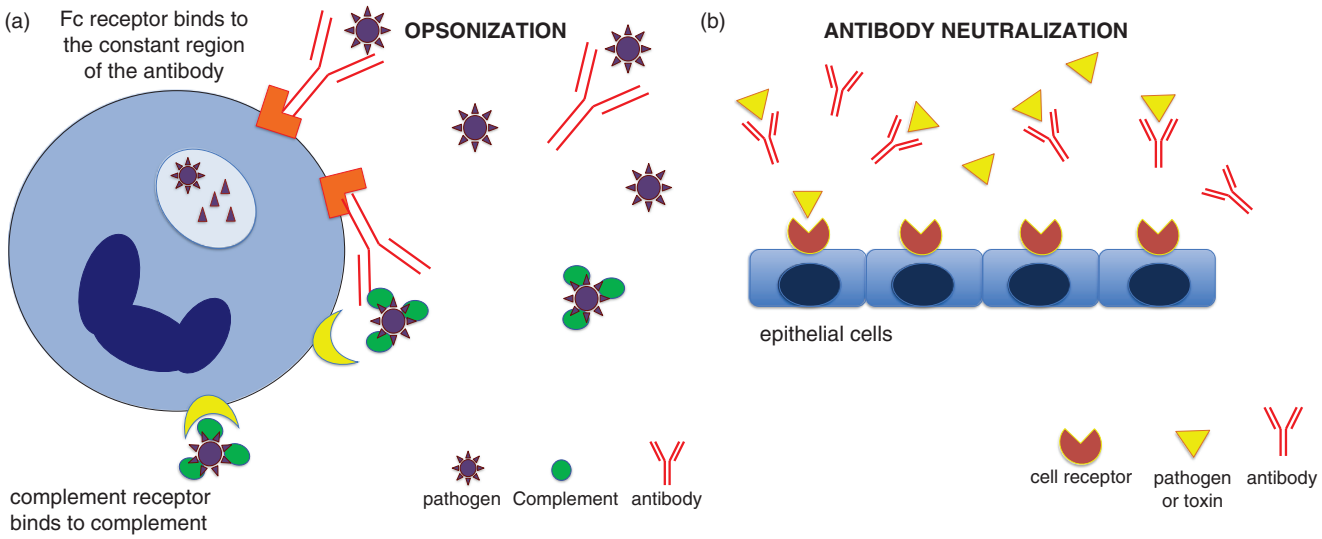

Figure 1.7 Opsonization and neutralization.

(a) Antibodies and complement opsonize (directly coat) antigens (e.g., pathogens, toxins). Neutrophils, macrophages and dendritic cells have antibody receptors on the cell surface (Fc-gamma and Fc-alpha receptors) that bind to the constant region of IgG and IgA antibodies, while their variable region binds to the antigen. These cells also have complement receptor. Once attached to these receptors, efficiency of phagocytosis is increased and, consequently, reactive oxygen and nitrogen species are produced for the destruction of pathogens. In other words, opsonization is essential for effective pathogen clearance. For encapsulated bacteria, opsonization with both immunoglobulin and complement is required for effective phagoctysosis and killing.

(b) Antibody-binding prevents pathogens or toxins to attach to cell surface receptors (neutralization effect).

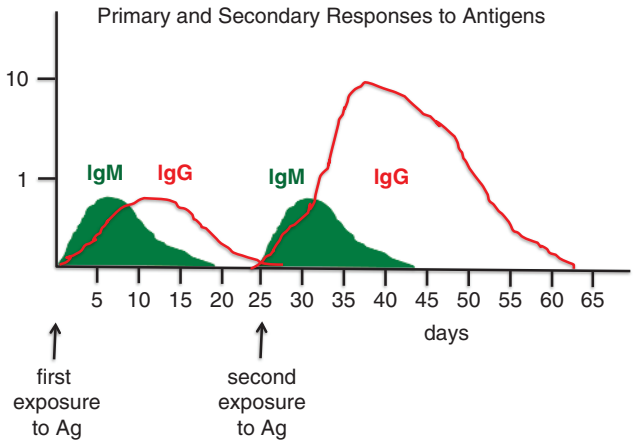

Figure 1.8 Primary and secondary immune responses.
Serum antigen-specific antibody concentrations serve to illustrate primary and secondary immune responses. First exposure to antigens induces initial population expansion and activation, with production of antibodies and memory cells. In a primary response, serum antigen-specific IgM concentration rises within a few days, and soon decays. Serum antigen-specific IgG concentration also rises, but requires a few more days to reach its peak, then levels decay. Upon a second encounter, a larger population of cells (memory cells) can recognize the antigen and respond quickly with antibody production. In a secondary response, again antigen-specific IgM rises quickly, but is now accompanied by IgG (faster response), which reaches much greater levels.

Therefore, the most significant impact in humoral protection is provided by IgG. However, other immunoglobulin isotypes broaden and reinforce immunity. Immunoglobulin isotype concentrations vary according to age, breed, and type of immune stimulation (type of pathogen, type of vaccine and adjuvant). Such differences may have implications for the quality and duration of protection, but overlapping of functions through the different immunoglobulin isotypes provides robust immunity.

In the horse, the immunoglobulin repertoire includes IgM, IgA, IgE, IgD, and seven different IgG isotypes, defined by different amino acid sequences in the heavy chain constant region. The most abundant of these are IgG_1 (formerly known as IgGa), $IgG_{3/5}$ (IgGT), and $IgG_{4/7}$ (IgGb), while IgG_2 and IgG_6 (IgGc) are found in smaller concentrations (their specific functions are unknown). IgG is present in blood, tissues and colostrum, and produced in large amounts in secondary responses (Figure 1.8). Colostral IgG is actively transported across the intestinal epithelium via the neonatal Fc receptor (FcRn). IgM molecules form pentamers, and are very efficient in activating complement.

IgM is the prevalent isotype in primary immune responses. IgA is produced in the lymph nodes and spleen in a monomeric form, and in the lamina propria of the MALTs in a dimeric form. In the latter, IgA binds to a receptor on the basolateral surface of the epithelial cells, and the receptor-IgA complex is taken up by endocytosis, moves toward the apical surface, and is finally secreted in the lumen. IgE is encountered in the blood and in tissues (e.g., skin, mucosa) already associated with mast cells, and is involved in allergic reactions.

Phagocytic activity is largely facilitated by immunoglobulin, which opsonizes foreign structures, and binds to immunoglobulin Fc receptors on phagocytes. In the absence of antigen-specific immunoglobulins, phagocytosis is largely inefficient; phagocytes may be present in large numbers, but cannot control infection by pathogen removal and killing – a scenario often observed in foals with low circulating IgG levels.

1.5 When the immune response goes wrong

Homeostasis is widely dependent on a protective and balanced response of the immune system, which involves redundant inflammatory and regulatory mechanisms. Occasionally, the immune system reacts to a non-self structure or a danger signal inadequately, either by not responding to it (immunosuppression or tolerance), or by over-reacting to it (autoimmunity). In these conditions, selective activation or suppression of specific responses becomes necessary.

Autoimmunity is an immune response against self-molecules involving B lymphocytes (antibodies) and/or T lymphocytes ($CD4^+$ helper or $CD8^+$ cytotoxic cells). Auto-reactive B and T cells are common to all individuals, but they may or not have an adverse effect in the body tissues with clinical manifestation. B and T cells are controlled by mechanisms of central and peripheral tolerance. However, immune dysfunction involving antigen-presentation and co-stimulation, genetic defects, and environmental factors may lead to autoimmunity.

The failure of the anti-idiotype control mechanism of antibody production may facilitate the circulation of autoantibodies: (an antibody is considered a new molecule (antigen) because of its variable region, and additional antibodies are developed against that variable region). In addition, molecular mimicry of microbes and self-epitopes may result in immune responses that overcome immunological tolerance and lead to tissue injury. Exposure of self-antigens present in systems that are not normally visited by lymphocytes (e.g., breakdown of the blood-brain-barrier or blood-ocular-barrier), or the development of new epitopes on self-proteins (e.g., infection with viruses, exotoxin damage of cell membranes, penicillin hapten binding to red cell membrane, or exposure to chemicals), can lead to the production of autoantibodies. In some cases, autoimmunity is associated with aging and immune system malfunction, or the effect of sexual hormones. The mechanisms that induce tissue injury are the ones described in hypersensitivity reactions.

Hypersensitivity reactions are inflammatory and damaging immune responses. The classification of hypersensitivity reactions is based on the type of cells and immune mediators that promote tissue injury:

(a) *Type I hypersensitivity reactions* (e.g., urticaria, insect-bite hypersensitivity, and food allergy) are mediated by antigen-specific IgE, mast cells, basophils and their mediators (see Chapter 4, Figure 4.1 and Chapter 5, Figure 5.2).

Table 1.1 Classification system of hypersensitivity reactions according to Gell and Coombs.

Type	Names	Definition	Examples
Type I	Immediate hypersensitivity	Mediated by IgE attached to mast cells in tissues or circulating basophils; reaction in minutes to hours; requires prior antigen sensitization	Anaphylaxis, Insect hypersensitivity, Food allergy
Type II	Antibody-dependent	IgG or IgM antibodies bind directly to cell membranes and cause tissue destruction via neutrophil chemotaxis and complement deposition; reaction in a few days	Immune-mediated hemolytic anemia, Immune-mediated thrombocytopenia, Pemphigus, Vasculitis, Glomerulonephritis
Type III	Antibody-antigen immune-complex	IgG or IgM form complexes with antigens, which deposit on cell membranes and cause tissue destruction via neutrophil chemotaxis and complement deposition; reaction in a few days	Purpura hemorrhagica, Vasculitis, Glomerulonephritis Serum sickness, Arthus reaction
Type IV	Delayed hypersensitivity, Antibody-independent	Mediated by CD4 T cells that activate macrophages, causing tissue damage or granulomas; delayed reaction in 2 to 3 days	Contact dermatitis Tuberculin test

prepared by Dr. Rolfe M. Radcliffe, Cornell University.

(b) *Type II hypersensitivity reactions* (e.g., immune-mediated hemolytic anemia or thrombocytopenia, pemphigus foliaceus, vasculitis, glomerulonephritis, drug hypersensitivity) involve auto-antibodies IgM or IgG against self cell-surface or extracellular matrix antigens. The antibodies function as opsonins, activating neutrophils and complement.

(c) *Type III hypersensitivity reactions* (e.g., purpura hemorrhagica, vasculitis, glomerulonephritis, serum sickness, Arthus reaction) are promoted by the random deposition of antigen-antibody immune-complexes in capillary vessels, with subsequent activation of neutrophils and complement (Chapter 8, Figure 8.2).

(d) *Type IV hypersensitivity reactions* (e.g., granulomas, tuberculin test, contact dermatitis) are mediated by sensitized $CD4^+$ T cells (Th1) and $CD8^+$ T cells (direct cytotoxic effect), which secrete inflammatory cytokine IFN-gamma that induces infiltration and activation of macrophages (Table 1.1; and see Chapter 13, Figure 13.3).

Immunodeficiencies are rare disorders of the immune system that result in failure to build protection against pathogens, leading to recurrent fevers and infections (Table 1.1; and see Chapter 21). These conditions may be transient or lasting, primary (genetic, inherited) or secondary (viral infections, immunosuppressive therapy, stress, endocrine disorders, nutritional deficiencies), and affect one or more arms of the immune system (humoral, cellular, and/or phagocytic systems). Described immunodeficiencies in the horse can be diagnosed using immunologic or genetic testing.

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