Περιεχόμενα:

Chapter 1

Neuroimmunology for the Non-Immunologist

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INTRODUCTION

It is not possible to describe immunology in a single chapter; however, neither **is it** necessary for a **clinical** neurologist to have detailed knowledge of immunology such as functions of specific cytokines, surface molecules, or signaling pathways. Instead, it is essential to understand concepts of how immune systems operate. This allows a clinician to understand when immune-mediated therapy is indicated, what it can or cannot do, when it may be necessary to choose aggressive immunosuppression, when broad immune suppression may be deleterious, and how other commonly encountered and treatable conditions, such as chronic

urinary tract infections, affect central nervous system (CNS) inflammation.

ROLES OF THE IMMUNE SYSTEM

To gain such conceptual understanding, it is necessary to look at the immune system from the evolutionary standpoint. The goal of the immune system is to mediate the survival of an organism and species. It accomplishes this goal through two related functions: (i) *protection of an organism from "foreign" or nonself entities, be they pathogens or mutated, neoplastic, or cancerous* cells,[1] and (ii) *wound healing*. These two intertwined functions have been evolutionary reinforced since the organization of life into complex cellular systems. The balance between ever-increasing diversity, complexity, and effectiveness of pathogenic infectious agents and the necessity of the multicellular organisms to simultaneously survive pathogen invasion and retain necessary self-functions (i.e., effective wound healing) are the forces that shaped the evolution of the immune system.[2] In contrast, autoimmune diseases (or even aging-related problems) were not the impediments an immune system had to struggle with over the past 500 million years; these problems are short-lived on the evolutionary scale. Therefore, we cannot understand the role immune system plays in these contemporary conditions if we do not first appreciate the functions immune system developed in response to evolutionary pressure exerted by pathogens.

The pathogen-driven evolution of the immune system over hundreds of millions of years contrasts with the short history of antimicrobial agents that started in 1928, with the discovery of penicillin.[3] A similar time frame was required for the evolution of wound healing, as organisms became more complex and required the rapid clearance of damaged tissue and formation of scars with strong structural integrity. It is only now, in the post-antibiotic era, that immune mechanisms such as fibrosis, with a strong evolutionary imprint, manifest themselves as deleterious. Fibrosis results in major disability, particularly in diseases associated with chronic inflammation and aging. It will be easier for us to fully understand and possibly reverse those deleterious functions of the immune system if we study them as positive forces in the survival of species.

EVOLUTION OF THE IMMUNE SYSTEM: INNATE AND ADAPTIVE IMMUNITY

Let's start with the essential function of the immune system, which is protection against pathogens: the most primitive immune systems (found in vertebrates, invertebrates, and plants) consist of virtually every cell of the body, but especially epithelial cells that line body surfaces, secreting protective molecules in response to injury or stress.[4] The most well-known examples include antimicrobial peptides called *defensins* and glycoproteins called *interferons*. The role of these secreted molecules is to directly suppress the invading pathogen and alert neighboring cells to the imminent danger. Signals that induce secretion of these molecules belong to two general categories: (i) *pathogen-associated molecular patterns* (PAMPs)[5] and (ii) *danger-associated molecular patterns* (DAMPs).[6] PAMPs represent evolutionary-conserved features specific to different types of pathogens and are generally not found in mammals. Examples of PAMPs that induce secretion of defensive molecules are double-stranded RNA or unmethylated CpG motifs found in certain viruses and bacterial components such as lipopolysaccharides, flagellin, and peptidoglycans. On the other hand, DAMPs represent molecules of the body that are not secreted into the extracellular environment under physiological conditions, such as orderly (apoptotic) cell death, but are readily released when a cell dies by necrosis (i.e., cell lysis often associated with lytic infections). Examples of DAMPS include purine metabolites such as ATP, chromatin-associated high-mobility group box 1 (HMGB1), and certain heat-shock proteins. Both PAMPs and DAMPs are recognized via ***pattern*** *recognition receptors* (PRRs). PRRs expressed on cell surfaces include *toll-like receptors* (TLRs),[7] *C-type lectin receptors* (CLRs),[8] and some *receptor kinases*.[9] PRRs can also be intracellular, recognizing pathogens that infected a cell or were ingested during phagocytosis; these include *NOD-like receptors* (NLRs)[10] and *RIG-I-like receptors* (RLRs).[11] Finally, there are also

secreted PRRs, such as *complement receptors, pentraxins* (e.g., serum amyloid and C-reactive protein), and *collectins,* such as *mannan-binding lectin* (MBL; which will be described in greater detail in the section on complement system).[11] These neutralize the pathogen by coating or opsonizing it, facilitating its uptake by the cells of the immune system, and also by activating complement, which can directly kill the pathogen.

As PRRs evolved in complexity (in response to the increasing diversity of pathogens and their effector and immune-evasion mechanisms), it became unsustainable for all cells of the organism to carry out these metabolically demanding immune functions. Thus, the next stage in the evolution of the immune system was development of cells specialized in sensing danger signals and responding to them. These now specialized cells and the panoply of aforementioned receptors and molecules became the initial or first-line defense against injury and pathogen entry and are collectively called the *innate immune system*.[12] The dominant group of cellular effectors of the innate immune system originate from single precursor cells called *myeloblasts,* giving rise to the name of the cells as being of *myeloid lineage*.[13,14] Examples of myeloid cells include most white blood cells that originate from bone marrow and are often short-lived, such as *neutrophils, basophils,* and *eosinophils* but also *monocytes* and *myeloid dendritic cells* (mDCs). Another myeloid lineage cell type are long-lived *macrophages* that are distributed in tissues and originate during embryogenesis from the yolk sac. These *tissue-resident macrophages,* different from monocytes derived from bone marrow, include *microglia* of the CNS,[15] *Kupffer cells* of the liver, *alveolar macrophages* of the lungs, and *Langerhans cells* of the epidermis. Newly generated monocyte-derived macrophages can replace some (e.g., alveolar macrophages) but not all (e.g., microglia) types of tissue-resident myeloid cells. The distinction between bone marrow-derived myeloid cells and tissue-resident, yolk-sac-derived myeloid cells may be very important for the pathogenesis of chronic inflammatory conditions, as these two cell types differ in their functional abilities and dominant signaling pathways. Specifically, most tissue-resident macrophages perform important homeostatic and tissue-repairing functions.[16] However, during chronic infection or autoimmunity, they can reprogram to a more pro-inflammatory phenotype, with a loss of their tissue-repairing functions, leading to an increase in organ damage.

The next evolutionary step was the development of a system of "adaptive" immunity.[17,18] In contrast to the innate immune system, which is "prewired" to respond immediately to external dangers, the adaptive immune system requires time to respond, but then acquires "immune memory," allowing rapid responses to previously exposed pathogens. The adaptive immune system further safeguards the organism against partially suppressed (although not necessarily eliminated) infectious agents such as human herpesviruses that may integrate their nucleic acid into the human genome. The adaptive immune system consists of new and more highly specialized cells, with new sets of receptors and mechanisms that generate a randomness and diversity necessary for fighting new or evolving threats. Quite amazingly, this evolutionary step likely utilized the invading pathogen(s), which provided a transposable element (retroposon) that conferred on the ancestral immunoglobulin-like gene the ability to undergo gene rearrangements mediated by recombination-activating genes (i.e., RAG1 and RAG2).[19–21] This allowed an organism to generate an unprecedented diversity of molecules that become "foreign recognition" receptors on the cell surface. These receptors are now called *T-cell* and *B-cell receptors* (TCRs and BCRs, respectively). Why was such diversity necessary? Because it allowed immune system to successfully deal with varied infections, including those that it never encountered before.

DIVERSITY (POLYMORPHISM) IS THE HALLMARK OF ADAPTIVE IMMUNITY

Diversity (polymorphism) is the hallmark of adaptive immunity, as it allows T- and B-cells to recognize, fight, and establish memory against diverse infectious or toxic threats. These highly polymorphic surface receptors recognize parts of infectious and noninfectious pathogens, which function as *antigens,* an antigen being any molecule that can interact with these cell surface receptors and result in stimulation of the cells. Antigens recognized by *TCRs* are usually

short peptides. There are two types of TCRs. Those composed of two glycoprotein chains called α (alpha) and β (beta) that cannot recognize antigens independently. Instead, these *α/β T-cells* can only recognize peptides that are bound to molecules called *major histocompatibility complex* (MHC) *proteins* expressed on the cell surfaces of other cells that present the antigen to T-cells. Such MHC-expressing cells then act as *antigen-presenting cells* (APCs). A different type of T-cell, called *γ/δ T-cells*, have TCRs composed of γ and δ chains.[22] These T-cells are less frequent in the blood than α/β T-cells. Rather, γ/δ T-cells reside predominantly in tissues, such as the gastrointestinal (GI) tract mucosa.[23] γ/δ T-cells have several peculiar features: instead of peptides, they tend to recognize lipid antigens, and they do not need MHC molecules to recognize and respond to these antigens. Finally, γ/δ TCRs have less diversity than α/β TCRs, and these TCRs tend to recognize evolutionary-conserved antigens that are common to several microbial organisms. Thus, γ/δ T-cells have features of both innate and adaptive immunity, and they carry out important immunoregulatory functions.[24] But let's return to the MHC system, which is necessary for activation of conventional, α/β T-cells.

In humans, MHC molecules are also known as *human leukocyte antigens* (HLA).[22,25] We recognize two major types of MHC molecules: *MHC class I* (human major alleles are called *HLA-A, -B,* and *-C*) and *MHC class II* (human major alleles are called *HLA-DR, -DQ,* and *-DP*). Analogous to the diversity of TCRs and BCRs, MHC molecules are also highly diverse. MHC molecules are inherited from both parents and do not have the potential to recombine and increase their diversity, as do TCRs and BCRs. Nevertheless, MHC proteins are sufficiently diverse to bind many different antigens, even those not encountered before. MHC-I molecules are expressed virtually on all cells of the body (some cells, such as neurons, may express MHC-I molecules in high quantities only during inflammation). MHC-I molecules bind peptides of 8 to 10 amino acids, derived generally from the *intracellular environment* (either pathogens that invaded a cell or normal self-peptides). In contrast, MHC-II molecules are expressed only on the cells of the immune system, specifically those that we call *professional APCs*, such as dendritic cells (DCs) and B-cells. MHC-II molecules bind longer peptides (13–25 amino acids) derived from proteins that are in the *extracellular environment* and are ingested (e.g., phagocytosed) and processed (broken down) by APCs.

Not every T-cell recognizes every MHC molecule. T-cells that express on their cell surface CD8 molecules are called *CD8 (cytotoxic) T-cells*, while T-cells that express CD4 molecules are called *CD4 (helper) T-cells*. This distinction is functionally relevant, because CD8-expressing T-cells display receptors that bind only to peptide-loaded MHC-I molecules (and thus recognize intracellular antigens), whereas CD4-expressing T-cells have receptors that bind to peptide-loaded MHC-II molecules (and thus recognize extracellular antigens). This dichotomy mediates different functions of CD8 and CD4 T-cells. However, some professional APCs can cross-present extracellular antigens to CD8 T-cells: this essential function for effective immunity (and likely also very important for autoimmunity) is carried by B-cells and certain DCs and macrophages.[26] We will return to functional differences between CD4 and CD8 T-cells later in this chapter.

DIFFERENTIATION OF B CELLS TO PLASMA CELLS: AFFINITY MATURATION AND ISOTYPE SWITCHING

In contrast to conventional T-cells that can recognize parts of proteins only when they are digested, processed to linear peptides, and loaded onto MHC molecules, *BCRs* can bind directly to proteins in their natural, 3-dimentional structure. Every BCR can only bind to a relatively small part of such a complex protein, and this small part is called a conformational *epitope*. *Antibodies* (Abs), also called immunoglobulins, are secreted by plasmablasts and plasma cells and are essentially the secreted and modified BCRs of the activated B-cell, which, by virtue of its activation, differentiated into an Ab-secreting cell.

Even though the BCRs are already diversified in the bone marrow by the same process that diversifies TCRs, Abs are further diversified, although not entirely randomly. Instead, diversification of Abs occurs as a result of increasingly limited concentrations

of the stimulating antigen: initially, when concentrations of stimulating antigen are high, B-cells with both high- and low-affinity BCRs for the antigen are stimulated. As antigen levels decline, low-affinity-BCRs are no longer stimulated, and there is a selective activation of B-cells with high antigen-affinity BCRs. This increasingly selective activation of high-affinity BCRs initiates a process of new BCR mutations.[27–29] Those B-cells that generated mutations that increased their affinity for the antigen receive a preferential BCR signal and survive. This process is called *affinity maturation* and occurs in secondary lymphoid organs, such as lymph nodes and spleen.[28] Parallel to affinity maturation, B-cells undergo another differentiation process called *isotype or class switching*. Abs come in different classes: the first class of Abs from recently activated B-cells (i.e., B-cells that did not yet initiate differentiation programs of affinity maturation and class switching) start producing is IgM Ab (sometimes called "germline" IgM). IgM Abs are usually low affinity Abs. To compensate for their low strength with which they bind an antigen, IgM Abs are pentameric—in other words, consist of 5 analogous Ab parts. They are produced at the beginning of an immune response. As activated B-cells continue to differentiate into long-living plasma cells and their BCRs increase their affinity for the antigen, these cells will also switch from the production of IgM Abs to monomeric immunoglobulins: IgG, IgD, IgA (secreted as dimer), or IgE Abs. IgD is not secreted but remains on the surface of B-cells as a BCR (all BCRs have the form of IgM or IgD monomeric immunoglobulins). These different forms of Abs (IgM, IgG, IgA, and IgE) are called "isotypes" and are determined by the heavy chain of the Ab body (see the following discussion).

Abs are glycoproteins belonging to the immunoglobulin superfamily and represent 10% to 20% of all plasma proteins. They play an important role in immunity and represent a bridge between innate and adaptive immune responses.[30] Structurally, they are complex proteins made of dimers of heavy chain and dimers of light chains. There are 5 types of heavy chains, corresponding to 5 isotypes of Abs: γ (corresponding to IgG), δ (IgD), α (IgA), μ (IgM), and ε (IgE). Each heavy chain can pair with one of the two light chains (κ or λ), and resulting dimers are assembled into a Y-shaped structure: the top of the Y contains two antigen-binding sites called *fragment antibody-binding* (Fab) fragments. The part of the Fab that undergoes mutations during Ab affinity maturation is called "hypervariable region" and represents amino acids that are in direct contact with the specific epitope of an antigen. On the other hand, the bottom part of the Y structure is called a *fragment crystallin* (Fc) fragment, and it largely determines the function(s) Abs elicit in the immune system.

IgG, the most common form of Abs secreted usually in highest concentrations, comes in 4 different "sub-isotypes": IgG1, IgG2, IgG3, and IgG4.[30] These sub-isotypes differ in some of their inherent functions, such as binding of the complement component C1q (IgG3 > IgG1 > IgG2 = IgG4). As we'll see later in this chapter, IgG sub-isotypes are produced in response to different types of pathogens. IgG (and all its sub-isotypes) and IgE are monomeric Abs but usually of much higher affinity for the antigen than pentameric IgM. The processes of affinity maturation and isotype switching require help from CD4 T-cells that recognize the same complex protein antigen as differentiating B-cells. Such a T-cell is called *follicular helper T-cell* (T_{fh}). These two processes (i.e., affinity maturation and isotype switching), which are the hallmark of B-cell differentiation to long-lived plasma cells, occur in the *germinal centers* of the *secondary lymphoid organs*, namely, *lymph nodes* and *spleen*. Thus, these B-cell/plasma cell differentiation processes are also called *germinal center reactions*.[28] Please note that some Abs (IgG2) undergo affinity maturation without the help of T_{fh} cells because they recognize nonprotein antigens.

The four main effector functions[30] of Abs are (i) *opsonization* (i.e., blockade of the epitope which interferes with its pathogenic roles; e.g., blocking proteins important for pathogen entry into cells or blocking secreted toxins), (ii) complement activation and resulting *complement-dependent cytotoxicity* (CDC), (iii) *phagocytosis* (i.e., internalization of pathogen to mediate antigen presentation and/or pathogen killing), and (iv) *antibody-dependent cellular cytotoxicity* (ADCC).

Opsonization is mediated by Fab fragments, and all Abs subtypes elicit this function. When Abs bind to self-antigens, they are called *auto-Abs*. Auto-Abs play an important role in CNS autoimmune diseases. Based on what we

discussed thus far, presence of high-affinity auto-Abs that bind protein antigen automatically implies the presence of autoreactive CD4+ T-cells that provide help to autoreactive B-cells. For example, the presence of auto-Abs against aquaporin-4 (AQP4) in neuromyelitis optica spectrum disorder (NMOSD) automatically implies the presence of AQP-4-targeting autoreactive CD4+ T-cells that may also actively participate in the disease process.[31] This will be further discussed in this chapter in relationship to immune tolerance.

The Fc fragment determines what happens to opsonized proteins: the Fc fragment of IgM and IgG Abs binds and activates the complement C1q component, which then initiates CDC. As previously mentioned, different subtypes of human IgG Abs bind complement with different strengths (IgG3 > IgG 1 >IgG2 = IgG4). Because IgG4 binds complement poorly, many therapeutic Abs that are designed to block a protein but do not kill the cells that harbors it are engineered to the IgG4 structure (e.g., natalizumab), whereas therapeutic Abs designed to kill the cells that carry targeted protein are usually engineered as IgG1 (e.g., alemtuzumab, rituximab, or ocrelizumab).

As we will see later in this chapter, the selection of the type of Abs secreted after the process of isotype switching is mainly determined by the target of the immune response; for example, viruses and bacteria elicit high levels of IgG secretion, especially of complement-binding IgG1 and IgG3 subtypes. On the other hand, helminths and other parasites generate IgE type of responses, capable of activating basophils and mast cells. IgA is mainly secreted at mucosal surfaces, so its production is elicited by mucosal pathogens. While protein targets elicit IgG1, -3, and -4 responses, which are dependent on CD4+ T_{fh} cell help, nonprotein antigens such as lipopolysaccharides elicit IgG2 type of responses without the need for T-cell help. Thus, whereas absence of one antibody subtype usually has no significant effect on immune responses (with exception of IgG1, which is the most prevalent subtype), the exception is IgG2 antibodies that bind nonprotein antigens often present in bacteria. Thus, selective IgG2 deficiency can predispose individuals to increased susceptibility to certain bacterial infections.[30]

IgG subclasses bind to a similar hierarchy (IgG3 > IgG1 > IgG4 > IgG2)[30] of Fc-receptors (FcR) expressed on the cells of innate immunity as they do to binding/activating complement. This determines the ability of IgG subtypes to elicit ADCC. However, this hierarchy does not necessarily imply that IgG3 or IgG1 Abs will cause ADCC in every instance. Because another immune cell is involved in ADCC (usually a natural killer [NK] cell but also cells of myeloid lineage). This "effector" cell is also sampling the environment and synthesizing positive and negative signals to determine whether it will, or will not, engage in ADCC. The positive signals include the numbers of Abs that engage FcR (thus targets that have higher expression on their cell surfaces usually cause stronger ADCC signals), the costimulatory molecules, PAMPs, DAMPS, and activating cytokines, whereas negative signals include normal expression of MHC molecules, *killer inhibitory receptors* (KIRs), and other inhibitory receptors and immunoregulatory cytokines. Because many of these regulatory signals are provided by tissues, the presence or absence of ADCC also depends on the tissue where the immune response is developing. In this regard, CNS tissue is quite special, normally providing strong immunoregulatory signals to killer cells. Consequently, some immunopathogenic IgG1 Abs that bind proteins expressed on neurons (such as anti-*N*-methyl-D-aspartate receptor Abs) do not cause CDC or ADCC unless the blood brain barrier is overtly dysfunctional or the aforementioned immunoregulatory mechanisms have failed. Instead, these Abs mediate neurological symptoms by target-blocking and target downmodulation from the cell surface. While this immunoregulatory function of CNS tissue is beneficial in protecting vital organs from immune-mediated damage, this tissue-specific property also limits effectiveness of cell-depleting therapeutic Abs: not only do these Abs reach cerebrospinal fluid (CSF) in concentrations corresponding to only 0.1% of serum levels, but the lack of complement and the lack/inhibition of cytotoxic cells in CSF/CNS leads to inadequate killing of intrathecal B-cells by monoclonal Abs such as rituximab, even if these are injected directly into CSF.[32]

In contrast to the clear hierarchy of IgG subtypes in binding C1q and FcR, binding to neonatal FcR (FcRn), which determines antibody half-life and the migration of antibodies to the placenta and bidirectional transport across mucosal surfaces (including CSF), does not

differ among IgG subtypes. Thus, the half-life of all IgG subtypes is around 21 days, and they all have similar CSF penetrance (and efflux).

The most dominant IgG subtype in the blood is IgG1, followed by IgG2, while both IgG3 and IgG4 subtypes are least prevalent. IgG3 responses tend to occur at the beginning of viral and bacterial infections (and IgG3 has the most pro-inflammatory role in the immunity), while IgG4responses tend to occur after many/repeated antigen exposures.[30] Elevated levels of IgG4 may play a role in several immune-mediated, often fibrotic diseases, including inflammation of the CNS. These are collectively called IgG4-related diseases.[33] Because IgG4 Abs are often formed in the absence of infection, they frequently represent the dominant subclass of Ab responses against therapeutic proteins, including therapeutic Abs.

Finally, we should also mention that in addition to Ab isotypes and IgG subtypes, there are also allelic variations of IgG subclasses called "allotypes," which are genetically determined. Some allotypes were found to be more immunogenic compared to others, which is important in development of therapeutic Abs (which should be least immunogenic) and may also be important in determining the phenotype of antibody-mediated autoimmune diseases. Similarly, Ab functions are affected by post-translational modifications such as glycosylation and fucosylation. By generating therapeutic monoclonal Abs in different expression systems, Abs with equal amino acid sequence may differ in their ability to mediate CDC and ADCC or have different half-lives.[34]

COMPLEMENT SYSTEM

The complement system belongs to the humoral part of innate immunity and augments (complements) innate immune mechanisms.[35,36] Like Abs, complement also opsonizes and thus neutralizes pathogens or their toxins and facilitates their antigen presentation and clearance by the cells of the innate immune system via phagocytosis. However, complement also interacts with the humoral arm of adaptive immunity, namely Abs, where aforementioned CDC represents important effector functions of Abs of IgM, IgG (mainly IgG3 and IgG1), and IgE subtypes.

The components of the complement system are produced mainly by the liver but also by some cells of the immune system (e.g., monocytes and macrophages, including microglia under physiological conditions when complement is used for pruning of synaptic connections between neurons)[37] and epithelial cells, including astrocytes, under circumstances of chronic inflammation.[38] Complement components are either soluble or membrane-bound proteins.

Under noninflammatory physiological conditions, complement components circulate in the blood in inactive form as pro-proteins.[35] Several different triggers activate enzymes called proteases, which activate one of the complement proteins. This activation sets up the cascade of reactions, which culminate in the formation of C5b-9 *membrane attack complex* (MAC) that makes a pore in the cellular membrane or outer membrane of the pathogen, leading to cell/pathogen death.

Complement can be activated in three different ways/pathways. First, the *classical pathway* requires Ab-antigen "immune complexes" and leads to activation of C1-complex. This pathway is best activated by pentameric IgM immune complexes or by several (ideally six) IgG immune complexes. Second, the *alternative pathway* can be activated under many circumstances, such as damaged cells or direct pathogen-mediated hydrolysis of the C3 complement component. Finally, the *mannose-binding lectin* (MBL) *pathway* is activated when MBL (part of the collections family), or its homologs called ficolins, bind to mannose residues (i.e., distinct sugar molecules) on the pathogens' surface. This complex then activates serum proteases (MASP1, -2, and -3), which cleave C4 and C2, fragments of which join together to form C3-convertase and activate the classical complement pathway. MBL binding to mannose residues also participates in the neutralization of viruses.

Complement activation by auto-Abs plays an important pathogenic role in several neuroimmunological diseases, especially NMOSD[39,40] but also some vasculitides[41] and possibly a subgroup of multiple sclerosis (MS) patients (i.e., patients with a pathogenic subtype of Ab-mediated demyelination, called subtype II) (see Chapter 3).[42] The activation of complement in these diseases can be demonstrated in pathological samples by presence of C9-neo-antigen (i.e., activated, cleaved C9) and,

diagnostically, in serum/CSF samples by presence of activated components of the MAC.[43]

To protect cells from inappropriate damage by complement, many regulatory proteins exist, both as soluble factors and membrane proteins.[36] Soluble C1 inhibitor and C4-binding protein inhibit activation of classical and mannose-binding lectin pathways, while soluble complement factor H (CFH) is the main inhibitor of the alternative pathway. Examples of membrane-bound complement regulators are MCP/CD46, DAF/CD55, CD59, CR1, and thrombomodulin. Thanks to the presence of membrane-bound regulators, MAC is not efficient in directly killing nucleated cells.[44] This is especially true if the antigen that an autoantibody targets is expressed sparsely on the cell surface (e.g., relatively low expression of CD25, targeted by monoclonal antibody daclizumab as compared to abundant expression of CD20, targeted by rituximab and ocrelizumab). However, sublethal injury caused by insertion of MAC into cell membranes of nucleated cells initiates a cascade of cellular events that may, depending on the circumstances, eventually lead to cell death. Thus, even when we consider a disease complement mediated, other immune mechanisms play important roles in modulating the pathogenicity of CDC. And, vice-versa, complement regulates activation of adaptive immunity, and activated T-cells express and secrete complement components.[35,44] Equally important to complement-mediated immunopathogenesis are the noncytolytic effects of sublethal MAC insertion into nucleated cells, such as release of chemokines and alarmins that exacerbate inflammation. Insertion of MAC to endothelial cells induces expression of adhesion molecules, activation of the clotting cascade, and vasodilatation[44] These complex interactions support the notion that the immune system skillfully integrates components of innate and adaptive immunity in achieving effective protection from pathogens and maintaining tissue homeostasis.

SELF-ANTIGENS, ANTIGEN PRESENTATION, AND IMMUNE SYNAPSE

We mentioned that under physiological conditions MHC-I molecules bind peptides derived from normal intracellular proteins and MHC-II molecules bind peptides derived from normal secreted proteins. These *self-antigens* (or *autoantigens*) do not usually induce activation of T-cells as a result of a phenomenon called *immune tolerance* (see following discussion). Nevertheless, self-antigens are (rather unexpectedly) required for survival of T-cells. In other words, every person has autoreactive T-cells. However, these T-cells normally do not cause tissue damage because they bind to autoantigens weakly (i.e., with low affinity) and their inadvertent activation is also kept in check by several complementary mechanisms of immune tolerance.

CD4 or CD8 molecules, together with the TCRs on the surfaces of T-cells form a complex with the peptide-loaded MHC-I or MHC-II molecules on the surfaces of the cells such as B-cells, macrophages, and monocytes. As noted previously, these cells will have processed the antigen into a form fitting into the two different MHC complexes, making them recognizable by TCRs. This process is called *antigen presentation,* and the cells doing the processing are called APCs. The interphase between T-cells and APCs is called the *immune synapse*. To form an immune synapse, both cells must stop (or, at a minimum, slow down) their respective movements. The information exchanged through the immune synapse is then processed by both cells, and based on this information, cells determine their next behavior toward each other.

How do T- and B-cells differentiate self-antigens from infectious antigens? The main difference resides in the context of antigen presentation. Because infections provide PAMPs (and often lead to tissue injury, which leads to secretion of DAMPs), the APCs are activated, which means that, in addition to an MHC-peptide complex for antigen presentation, they also express on their cell surfaces many costimulatory molecules, as well as secreting cytokines that further activate primed T-cells. Thus, in the process of T-cell activation, the MHC-peptide complex is called *signal 1*, the co-stimulatory signal (provided by many different binding partners, such as CD80-CD28, CD86-CD28, CD40-CD40L, ICOS-ICOS-L, and many others) is called *signal 2*, and the cytokine activating signal is called *signal 3*. Signals 2 and 3 are provided by APCs only during their activation (usually by pathogens). Naïve T-cells

(i.e., cells that have not been fully activated yet) require all 3 signals to enter the proliferation cycle and become fully activated. Because co-stimulatory signals and pro-inflammatory cytokines are upregulated on APCs in response to PAMPs or DAMPs, under physiological conditions activation of naïve cells occurs only under conditions of infections or tissue injury. T-cells that have been activated previously have lower requirements for co-stimulation than naïve T-cells. Recently activated T-cells are called *effector* T-cells, while T-cells activated a long time ago (and those who survived the contraction phase of the immune response and became deactivated) are called *memory* T-cells. These effector and memory T-cells have a *lower activation threshold*, which means that they can become activated by smaller concentrations of antigens combined by weaker co-stimulation. Still, these cells usually require some combination of co-stimulatory signal and pro-inflammatory cytokines to become fully activated under physiological conditions. Their excessive or inappropriate activation is regulated by processes of central and peripheral tolerance described in the following discussion. Because of the multiplicity of positive and negative signals that govern activation of immune cells, common genetic variants, each effecting only one of these myriads of molecules marginally, can collectively lower the activation threshold of immune cells substantially and, as a result, make the subject prone to autoimmunity. This is true for polygenic autoimmune diseases such as MS and systemic lupus erythematosus, among others. On the other hand, rare genetic mutations of essential immune-regulatory molecules can cause systemic autoimmunity or organ-specific immune-mediated tissue injury, including inflammation of the CNS. The examples of such monogenic immunodeficiencies affecting CNS are perforin deficiency (which can present as isolated CNS inflammatory disease or as hemophagocytic lymphohistiocytosis [HLH] with or without CNS involvement)[45] and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) mutations.[46]

IMMUNE TOLERANCE

The harmless behavior of T-cells to self-antigens is assured by T-cell "education" in the thymus, which we call *thymic selection*.[47] Thymic selection has two parts: *positive* and *negative selection*. Positive selection means that T-cells that have generated (through random recombination) TCRs that do not recognize any self-antigens bound to either MHC-I or MHC-II molecules die in the thymus by "neglect." In other words, self-antigens must provide survival signals for immature T-cells to be positively selected and survive this first education step. Positively selected T-cells are then subjected to negative selection: those T-cells that reorganized their TCRs so that they bind to self-antigens presented by MHC-I or MHC-II alleles too strongly are killed by apoptosis, because they are deemed dangerous. They might attack normal cells of the body if they got out of the thymus. This negative selection process is also called *central tolerance* or *thymic tolerance*.[48] Defects in this process—for example, due to inherited or new (de novo) mutation(s) in genes that code essential proteins that regulate this process, such as forkhead box P3 (FoxP3)—underlie the development of generalized autoimmunity. A similar process of central tolerance educates immature B-cells in the bone marrow and spleen.[49] Because of the essential role the *thymus* and *bone marrow* play in the development and education of cells of the adaptive immune system, they are called *primary lymphoid organs*.

Finally, those T-cells (and B-cells) that reach the blood are also kept in check to make them nonresponsive to self-antigens by multiple mechanisms that together constitute *peripheral tolerance*.[50] There are many mechanisms of peripheral tolerance, including regulation of one type of immune cell by others (i.e., *regulatory T-cells, regulatory B-cells,* or *regulatory NK cells*). T-cells and B-cells can also be inactivated by a process called *anergy*. The two essential regulatory molecules participating in peripheral tolerance are CTLA-4 and PD-1.[51] We mentioned in previous section that CTLA-4 mutations lead to inflammatory disease that commonly affects CNS tissue.[46] Inhibitors of the PD-1 pathway (also called "checkpoint inhibitors") are highly effective cancer drugs, as they facilitate autologous immune responses against cancers. However, they commonly unleash autoimmune responses, often affecting the CNS.[52] With the exponential growth of these immune-boosting therapeutic strategies for cancer, neurologists will be encountering