Review Article

Immunology primer for neurosurgeons and neurologists part I: Basic principles of immunology

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Abstract

Our knowledge concerning the workings of the immune system has evolved considerably over the past 20 years, with great strides being made as regard to complex interactions and repertoire of effector reactions under a host of conditions. Many of our previous understandings have been challenged, especially concerning tumor immunology and autoimmunity. Also of critical importance is our understanding of how the immune system terminates its attacks and the mechanisms used to regulate the balance between proinflammatory and antiinflammatory mechanisms, so as to prevent excessive immune bystander damage. I will discuss in part I the basic physiology of innate immune function and the immune systems reactions to invasion by microorganisms.

Key Words: Adaptive immunity, cell-mediated immunity, cytokines, innate immunity

INTRODUCTION

The immune system is the guardian of the body, protecting it from harmful invading organisms, toxic environmental chemicals, cellular debris, and cancer development. Next to the nervous system, it is the most complex system in the body and comprises a great number of tissues, organs, cell types, and secreted molecules, which interact in a very complex manner, providing a dynamic, highly versatile and, in many instances, a very specific defense. It should also be appreciated that it is intimately connected with and interacts with other systems, especially the nervous system and the endocrine system.

Over the past two decades we have learned a great deal about the immune system, which has changed much of our thinking concerning not only how the immune system operates, but also the very nature of many immune disorders, including autoimmunity, neurodegenerative diseases, and the importance of immune balance. For instance, we now view autoimmunity not as a misdirected attack by a normally functioning immune system against “self” proteins, but rather a broad scaled attack by immune molecules triggered by a dysfunctional immune system. Newer evidence is clearly demonstrating a powerful link between immune mediators and excitotoxicity, which I have called “Immunoexcitotoxicity”.

One should appreciate that neurotransmitters and endocrine molecules, such as catecholamines, acetylcholine, and glucocorticoids, also regulate and control immune reactions. Dysfunction of these neuroendocrine and neurotransmitter systems can significantly contribute to chronic inflammation of the brain and eventually to a number of neurodegenerative
disorders. For example, disruptions in all three systems (neuroendocrine, neurotransmitter, and immune systems) are seen with Alzheimer’s disease.

Until recently, the brain was considered an immunologically privileged site, based on the fact that most antibodies and immune cells are not found within the normal brain parenchyma. The blood–brain barrier (BBB) plays a major role in protecting the brain from acute invasion by immune cells and certain immune mediators.

We now know that the brain not only has its own innate immune system, composed of microglia, astrocytes, and mast cells, but it can also recruit an array of peripheral immune cells, mast cells, monocytes/macrophages, eosinophils, and lymphocytes into its substance by way of areas having an open BBB, called the circumventricular organs (CVOs). This usually occurs with intense immune activation or chronic systemic inflammation.

Under pathological states, entry of peripheral immune cells into the central nervous system (CNS) activates microglia and astrocytes at the sites of entry and these activated microglia then travel great distances throughout the CNS.

Compelling evidence now suggest that activation of the peripheral immune system rapidly activates the innate immune system (within minutes) within the CNS and this can have profound effects on neurodevelopment, brain aging and the risk of developing a neurodegenerative disease, such as Alzheimer’s, Parkinson’s disease, or amyotrophic lateral sclerosis (ALS).

With direct invasion of the brain substance by pathogenic microorganisms, the BBB often becomes dysfunctional and antibodies, cytokines, chemokines, interferons, and leukocytes enter the CNS in great numbers.

We see this with bacterial meningitis and encephalitis. The mechanism by which the brain attracts these immune components involves a complex interaction between the innate and adaptive immune systems.

Systemic infections can also have profound effects on the outcome of a number of neurological conditions and affect how the brain functions in terms of behavior, learning, memory, attention, and language, something we call sickness behavior. The link between systemic inflammation and activation of the brain innate immune system occurs by several routes, each of which determines the speed of innate CNS immune activation and duration. Newer evidence suggests that activation of the systemic immune system, especially if prolonged or intense, can dramatically affect CNS neuropathology, especially when microglia are primed. This may play a significant role in chronic traumatic encephalopathy.

Immune reactions within the brain also explain what has been referred to as “febrile seizures”, which, in my opinion, are in fact immunoexitotoxic seizures, involving proinflammatory cytokines interacting with specific glutamate receptors. High fevers are accompanied by significant elevations in proinflammatory cytokines, such as IL-1β, TNF-α, and IL-6, with IL-1β playing the predominant role. Elevations in all three of these immune mediators have been associated with seizures. One also sees a concomitant elevation in regional brain glutamate, which plays a major role in febrile seizures. The fever itself may be playing a secondary role in enhancing neuronal sensitivity to immunoexitotoxicity.

Of great interest recently is what has been referred to as bystander damage, that is, injury to the surrounding tissues caused by the immune reaction itself. This damage can extend quite a distance from the primary immune reaction, as we witness in the brains of multiple sclerosis patients. For example, magnetic resonance imaging (MRI) scans do not demonstrate new lesions in many cases of worsening symptoms or MS progression, but positron emission tomography (PET) scans demonstrate a much wider zone of inflammation than is seen on the MRI scans. Likewise, microglial activation is known to occur distant from immune reactions to Abeta (explain as beta amyloid), explaining much of the observed tissue damage distant from the plaque accumulations themselves.

We will also see that the outcome of major infections are linked to things that previously were not considered “immunological”, such as various neurotransmitters, and that a growing number of environmental chemicals, such as pesticides/herbicides and fungicides, can alter immune function and hence the outcome of common infections both by affecting immune cells themselves and by affecting neurotransmitters.

Of equal importance, the brain can affect not only overall systemic immune function, but also individual components of the immune system.

This is of particular importance in chronic stress and explains how such things as depression are linked to atherosclerosis, heart attack, and stroke risk. That is, research and clinical studies have strongly linked chronic inflammation to the development and acceleration of atherosclerosis and likewise have linked chronic inflammation to major depression. It is known that those having suffered a myocardial infarction and also having major depression are more likely to have a fatal recurrence of their myocardial infarction than nondepressed patients. Elevated inflammatory markers are frequently found in both conditions and precede the original myocardial infarction. Elevations in extraneuronal glutamate within limbic structures are also related to major depression and in experimental studies elevations in blood glutamate levels chronically increase endothelial free radical generation and lipid peroxidation, conditions associated with aggressive atherosclerosis. A number of new terms will be discussed that have entered the lexicon of the immunologists.
In this discussion, I will cover the basic understanding of immunology and some of the newer discoveries concerning the cellular and molecular aspects of immunity one needs to understand these complex reactions.

THE ANATOMY AND PHYSIOLOGY OF THE IMMUNE SYSTEM

Systemic immunity
The systemic immune system consists of a number of cell types, either fixed within tissues or floating freely within the extracellular fluid spaces and vascular system (blood vessels, reticuloendothelial system, and lymphatics) and a growing number of immune molecules secreted from these cells and tissues (cytokines, chemokines, interferons, eicosanoids, etc.). Within fixed tissues, they act as gateway guards to prevent entry or transfer of harmful microorganisms or toxic products into the body. Examples of these guardian cells include dendritic cells, mast cells, hepatic Kupffer cells, renal mesangial cells, serosal macrophages, brain microglia, endothelial macrophages, and alveolar macrophages. Some of these can also wander and be attracted to nearby sites of microbial invasion or tumor growth.

Since most invading organisms enter the body either via the skin, respiratory mucosa, genitourinary mucosa, or the mucosa of the gastrointestinal tract (GI tract), it is in these locations that we find the majority of immune tissues (80% are within the GI tract). And while we tend to think of the “immune system” as consisting of specific tissues (lymph nodes, spleen, reticuloendothelial system, etc.), we must also recognize the importance of tissue barriers, such as, skin, cell membranes, connective tissues, and cell basement mucopolysaccharides, as they also guard against tumor and microorganism invasion. In many cases connective tissue barriers encase sites of infection or chronic inflammation so as to prevent further spread into surrounding tissues.

While in general immune activation in the CNS shares many of the immune mechanisms seen in the rest of the body, it also has special mechanisms as well. This is because of the sensitive nature of postmitotic neurons in the CNS as well as its many neural pathways. In the past, the CNS was considered to be an immunologically privileged site, but now we know that this is incomplete.

Typical immune cells, such as lymphocytes, eosinophils, basophils, and plasma cells, are not normally found in the CNS parenchyma. The primary barrier is the tight endothelial junctions of the BBB, which restrict entry. There are several methods of entry during infections and with brain ischemia/hypoxia and trauma that will be discussed later. During active brain infections, entry of mass number of neutrophils, macrophages, mast cells, and lymphocytes occur quite rapidly. This is mainly secondary to disruption of the BBB, but also from mass entry through the CVOs, which includes the choroid plexus, subfornical organ, organum vasculosum of the lamina terminalis, median eminence, posterior pituitary, subcommissural organ, and the area postrema. For example, neutrophils enter in large numbers via the choroid plexus during bacterial meningitis.

The brain not only has its own innate immune cells, primarily the microglia, but also astrocytes and endothelial cells. In fact, microglia, astrocytes, endothelial cells, and even neurons can release cytokines during disturbances of CNS homeostasis. Both microglia and astrocytes have an extensive network of foot processes, which surround the neuron and synapse and are in close opposition to the vascular network of the BBB. This allows a constant sampling of the extracellular fluid, allowing early detection of cytokines and other immune mediators from the systemic circulation. For example, systemic IL-1ß can rapidly activate brain microglia.

Divisions of the immune system- A general overview
Basically the immune system is divided into an innate immune system and an adaptive immune system, with some overlap in function [Figure 1] [1st slide—innate and adaptive immunity].

1. The innate immune system, along with the various tissue barriers, can be thought of as the first line of defense and it requires no previous exposure to the invader for its defensive functions. In essence, it is a nonspecific defense system.

2. The adaptive immune system is more specific and must use a number of methods to identify the foreign invader’s unique signature (antigens) and then assign specific cells and cell products, such as antibodies, interferons, and cytokines, to neutralize...
the invaders and record their presence for future defense in specialized memory cells, should another attack by the same invader occur later.

In most cases these two arms of the immune system operate in tandem yet there is a great deal of interaction necessary for optimal immune defense, that is, the innate immune system enhances the reactions and prepares for the adaptive immune reaction. It should be appreciated that the most successful defense is when the invader is prevented from reaching such large numbers that the immune system would eventually be overwhelmed, meaning that the early defense mechanisms are critical in keeping microbe titers low. In most cases, the immune components on the surface of the mucosa, such as IgA antibodies, neutralize the potential invader, thus preventing any entry from taking place. This circumstance occurs commonly in people who never seem to get sick from a virus.

Studies have shown that those with impairment of the innate immune system suffer frequently from overwhelming infections, since the impaired innate system allows rapid replication of the organisms. Experimental studies have also shown that animals with an intact innate immune system, but without adaptive immunity, can survive infections from pathogenic organisms initially, but eventually the organism will overwhelm the animal.

The immune system systemic in most mammals is composed of an array of immune cell types, immune secretions, lymphatic channels, lymph nodes, the spleen, and the reticuloendothelial system. Lymphatic channels are present in every tissue and organ, except the brain and spinal cord, which has its own extracellular circulatory system. It is important to appreciate that extracellular fluids from the brain do drain into the cervical lymphatics and have access to the cervical lymph nodes so as to utilize the immune cell activation mechanisms within the peripheral lymphatic tissues. This allows immune processing of molecules from the CNS by specific immune cells, which can then return to the brain as fully activated mast cells, lymphocytes, or macrophages. Peripheral macrophages become brain microglia once inside the CNS parenchyma. Functionally, macrophages and microglia are virtually identical.

The primary lymphoid tissues include the thymus, fetal liver, and bone marrow, where lymphocytes and monocyte/macrophages are produced. T-lymphocytes (T-cells) are produced in the bone marrow and mature in the thymus gland and B-lymphocytes (B-cells) are produced in the fetal liver or bone marrow [Figure 2] [3rd slide—development of T-cells in the thymus]. Secondary lymphoid organs include the spleen, lymph nodes, and mucosal surfaces such as tonsils and Peyer’s patches in the gut. Most lymphocytes reside within lymphoid tissue.

It is in the secondary lymphoid organs that immune interactions take place such as reactions with antigen presenting cells (APCs). The spleen contains specific regions devoted to both T- and B-cells and is characterized by an intimate connection with the circulation. Lymph nodes resemble the spleen in that they have a rich blood supply, afferent and efferent lymph channels and segmental compartmentalization of T- and B-cells. The mucosal associated lymphoid tissue (MALT) is a dispersed aggregate of nonencapsulated lymphoid tissue found especially in the submucosal GI, respiratory, and urogenital tracts.

The APCs, such as the dendritic cells (which include microglia) located within the parenchyma of tissues and organs are activated by antigens through immune receptors on their surface membranes; the cells are then transferred to the lymphoid tissue, where they present the invading antigen to the lymphocytes and other immune cells [Figure 3]. This process activates the effector cells, such as the phagocytes and lymphocytes, which then arms the innate and adaptive immune systems. The dendritic cells, by being incorporated within the tissues themselves, especially at sites of invader entry, such as the skin, mucosal linings, and BBB, allow rapid immune responses to take place.

The innate immune system in response to an immediate attack

As for the innate immune system, the antigen activates the receptors on the cell surface and endoplasmic reticulum quickly, without the prolonged activation process necessary for adaptive lymphocyte activation, which allows a more rapid reaction time [Figure 4]. The surface receptors on the immune cells, principally neutrophils and macrophages, activate cell signaling that enhances inflammatory processes, and the release of type I interferons (IFN-1α and IFN-β). The type I interferons suppress viral replication, thus giving the adaptive immune system time to mount highly specific humoral and cell-mediated immune reactions against the virus. Interestingly, type I interferons also suppress excessive immune reaction, so as to minimize bystander damage to surrounding normal tissues. We see this interaction between proinflammatory and antiinflammatory/ reparative systems in both the innate immune reactions and adaptive reactions. This emphasizes the real danger to tissues, especially those of the CNS, from excessive immune reaction.

Immune receptors and classical activation of the innate immune system

The innate immune system can recognize 10⁵ molecular antigenic patterns, whereas the adaptive immune system can recognize 10⁵ to 10⁶ distinct antigens. This differential makes sense, as the main function of innate immunity is to recognize foreign invaders and reduce their numbers, mainly by phagocytosis and some direct killing. The adaptive immune system can differentiate between many subtypes of the same organism and has the goal of elimination of the surviving microbes.
Pathogen recognition is required for both the innate and the adaptive immune system
Pathogen recognition is accomplished by a complex series of receptors located both on the surface membrane and within the cytoplasm of immune cells. These receptors respond to two major classes of molecular signals—pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [Figure 5]. PAMPs include a variety of microbial products such as double-stranded RNA, N-formylmethionine, mannose-rich oligosaccharides, lipopolysaccharide (from Gram-negative bacteria), and lipoteichoic acid (from Gram-positive bacteria). DAMPs recognize products of cell damage and use different cell signaling mechanism than do PAMPs.

The PAMPs and DAMPs bind to cell receptors called pattern recognition receptors (PRRs), which are linked to cell signaling pathways such as MyD88, NFκB, and IRFs (interferon response factor for release of type I interferons). These cell-signaling pathways regulate inflammatory genes and control the generation and secretion of cytokines and other immune molecules in response to foreign antigens. In addition, they regulate the generation and secretion of adhesion molecules such as selectins and integrins, which regulate leukocyte transmigration through vessel walls, thus allowing immune cell migration to areas of inflammation.

There are a number of PRRs. The major PRRs include toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-like receptors (RLRs), G-type lectin-like receptors (such as mannose receptors), and scavenger receptors (such as CD36). Each of these receptors reacts with a specific set of ligands, many from bacteria, fungi, viruses, and mycobacteria such as lipopolysaccharide, peptidoglycan, flagellin, and microbial nucleic acids [Figure 6]. Recent studies have shown that with many antigens several TLRs and other receptors act in concert, thus increasing the complexity and specificity of reactions by the immune system.
The NLRs are a family of cytoplasmic proteins that when stimulated form signaling complexes that promote inflammation called NLRP inflammasomes. The NLRP inflammasomes respond to a wide variety of cytoplasmic stimuli, such as microbial products, crystals (urate), and aluminum. PAMPs and DAMPs activate these inflammasomes within the cell.

Thirteen TLRs have been identified with various locations on the cell surface as well as inside the cell. For example, TLR 2 and TLR 4 are found on the surface membrane and TLRs 3, 7-9 are found on the membrane of the endoplasmic reticulum [Figure 7]. The cytoplasmic TLRs allow the immune system to react to viruses and bacteria within the cell. Dendritic cells have the widest variety of PRRs. They are also abundant on neutrophils and macrophages.

General principles of immune cells and soluble recognition molecules

Immune cells

Cells making up the immune system can be fixed in tissues, but are mostly characterized by an ability to move about in the extracellular spaces, blood, and lymphatic fluids. This mobility allows them to collect in zones of homeostatic disturbance, such as seen with tumor development, infectious invasion, or injury. Immune cells are referred to as leukocytes and include neutrophils, basophils, eosinophil’s (collectively called polymorphonuclear cells), monocytes/macrophages, and lymphocytes. There are a growing number of specific subtypes for each cell type.

In general, at the site of microbe invasion or tissue injury, one sees a release of chemoattractants called chemokines, which attract immune cells to the site of the inflammation. Before describing this process, I will first describe the various cells participating in the inflammation process.

Soluble recognition molecules

In addition to immune cells, the immune system also manufactures a number of soluble molecules that exist in the plasma and extracellular fluid spaces. These products aid in the phagocytosis and occasionally, direct killing of microbes.

These include:
- Pentraxins in the plasma, such as C-reactive protein.
- Collectins in the plasma and pulmonary alveoli
- Ficolins in the plasma
- Complement in the plasma
- Natural antibodies, such as IgM in the plasma

Immune cells: Specific functions

Polymorphonuclear granulocytes
Granulocytes (polymorphonuclear leukocytes [PMNs]) are produced in the bone marrow and are the most abundant immune cell in circulation, with over 80 million PMN cells being produced every minute. They make up some 60-70% of circulating leukocytes. Unlike lymphocytes and monocyte/macrophages that can live for months to years, PMNs survive only 2-3 days. Different from other immune cells, PMNs are not dependent on antigen/antibody reactions, but rather protect by phagocytosis of invading organisms or tumor cells. Their phagocytic activity is enhanced by activation of cell surface receptors and interaction with opsonins.

They have a unique ability to penetrate the walls of blood vessels and other tissues, giving them ready access to invading organisms, tumor cells, and tissue injury sites. Significant deficits in PMN numbers are associated with severe states of susceptibility to infections.

Neutrophils

Over 90% of granulocytes are of this cell type. They are characterized by two types of intracellular granules: One a lysosomal granule containing acid hydrolases, myeloperoxidase, and murminidase and another that contains lactoferrin and lysozyme—enzymes that destroy the substance of invading organisms and tumor cells. The central killing method of neutrophils is by the
enzyme NADPH oxidase, which generates high levels of superoxide, a reactive oxygen species (ROS). Superoxide itself is a rather weak radical, but readily combines with nitric oxide, also produced by neutrophils, to form a very powerful reactive nitrogen species (RNS) called peroxynitrite. Also produced are high levels of hydrogen peroxide, which breaks down into the more powerful ROS hypochlorous acid, hydroxyl radical, and singlet oxygen, all of which are powerful antimicrobial compounds.

When organisms or tumor cells are ingested, they are contained within special vacuoles called phagosomes and these then fuse with the enzyme-containing granules to form a phagolysosome. It is within these compartments that both enzymatic action and high levels of nitric oxide and hydrogen peroxide kill the organism or tumor cell.

Like microglia, neutrophils also undergo a process of “priming” in which the cell is not fully activated, but defensive mechanisms such as adhesion molecules, phagocytosis, production of ROS, cytokine secretion, leukotriene synthesis, and degranulation are all enhanced, but actual bactericidal processes are not yet triggered. Priming agents promote efficient clearing of invading microorganisms or tumor cells, in part by acting as TLR agonists. These are activation receptors found on the surface of the APCs—that is, immune cells that present the antigen to the main attacking immune cell, such as lymphocytes and macrophages.

To facilitate phagocytosis the immune system resorts to the use of opsonins (Greek, “to prepare for eating”), substances that help bind the invader to the immune cell using phagocyte receptors. Opsonins can include antibodies, complement molecules, and fibronectin from the basement membrane. Neutrophils, and other immune cells, recognize a great many surface-bound or secreted bacterial viral and tumor cell components, called PAMPs, which react with PRRs on the phagocyte’s surface. This allows these immune cells to correctly identify the invader, thus avoiding mistaken identity problems.

Neutrophils also contain special TLRs that can extend the life of the cell and enhance adhesion, phagocytosis, release of cytokines and chemokines, and ROS, making these immune cells much more efficient. The azurophilic granules within the vacuoles of neutrophils contain special antimicrobial peptides (AMPs) within the phagosome, which include β-defensins, cathespins, proteinase-3, elastase, and azurocidin. The most abundant of the AMPs are β-defensins. At least some of the defensins are generated by vitamin D3. These AMPs can directly kill invading organisms and may play a role in protecting against tumors as well.

**Eosinophils**

In nonallergic, healthy individuals, eosinophils make up approximately 2-5% of circulating leukocytes. While they can engage in phagocytosis, it is not their primary function. One of the characteristics of these cells is the dense packing of eosinophilic granules in the cytoplasm, which, when activated, degranulate, fuse with the cell membrane, and are then released. These cells are especially useful against large targets, such as parasites.

**Basophils**

These cells are found in very small numbers within the circulation. They are characterized by the presence of deep blue granules on staining their cytoplasm and have many properties quite similar to mast cells. Like mast cells, they contain heparin, slow-reacting substance-A (SRS-A) and eosinophilic chemotactic factor for anaphylaxis (ECF-A), which are released by appropriate stimulation. They are associated with the common symptoms of allergies and may be effective against parasites as well.

**Mast cells**

These specialized secretory cells are mainly found throughout the connective tissues of the body, but are also found within the brain as well. Normally, they are not seen in the blood stream, but they can enter the brain from the blood during brain injury and during development of primary brain tumors. They are characterized histologically by large secretory vesicles. While most are aware that mast cells secrete histamine, in fact, they secrete a number of biologically active compounds, including leukotrienes, prostaglandins, platelet activating factor, and proteolytic enzymes (plasmin, hydroxylase, β-fluorocoridase, and phosphatase).

Studies have shown that mast cell invasion of gliomas occurs as an early event and the heaviest infiltration is seen in the more malignant tumors. Most studies have shown that dense populations of mast cells in a tumor worsen the prognosis, and may be immunosuppressive.

They play a major role in type 1 (immediate) hypersensitivity reactions. It is the mast cell that is the major player during anaphylaxis, with the release of histamine, proteases, and proteoglycans (heparin). It also activates other inflammatory cells during such reactions, including basophils, eosinophils, and Th2 cells. Mast cells are increasingly being seen to play a role in inflammatory brain disorders.

**Plasma Cells**

Plasma cells are responsible for synthesizing antibodies and are differentiated from B-lymphocytes. Plasma cells are distinguished from B-cells by a lack of surface complement and immunoglobulin receptors. They tend to gather at sites of antigenic accumulation, that is, zones of invasion or tumor development. These cells live from days to months and can secrete antibodies at a rate from hundreds to thousands per second per cell.

**Monocytes/macrophages**

Monocytes are derived from the bone marrow and are
the precursor of macrophages, which are found virtually everywhere in the body, including the brain. When monocytes leave the blood and enter tissues they are transformed into macrophages, which are characterized by phagocytic activity and the local release of toxic compounds, including glutamate, in high concentrations. Because macrophages are located along the endothelial surfaces of blood vessels, on mucosal surfaces and other surfaces facing the external environment, they constitute a first line of defense. Macrophages can also act as antigen-presenting cells (APCs) by presenting antigens to the lymphocytes for identification.

Macrophages contain a number of surface glutamate receptors and activation of these receptors play a role in tissue damage during prolonged immune activation, especially in the brain and spinal cord. Glutamate is converted to glutamate by mitochondrial glutaminase enzyme and therefore plays a role in immune function as well. As mentioned, macrophages can readily enter the brain and spinal cord, transform into microglia, and as microglia release an assortment of cytokines, chemokines, interferons, and a number of excitotoxic molecules, thus initiating immunoexcitotoxicity. There is evidence that glutamate secreted from a number of immune cells, plays a significant role in neurodegeneration and CNS autoimmune disorders, such as multiple sclerosis.

Lymphocytes

Lymphocytes play a major role in immunity as immune “recognition cells”. These cells are derived from stem cells in the lymphoid organs and some migrate from the circulation to the spleen, lymph nodes and unencapsulated lymphoid tissue. The average adult has about 10^12 lymphoid cells or 2% of the body weight. Lymphocytes make up about 20% of the total leukocytes. Because they are needed for immune memory, many lymphocytes live for several years.

There is a good deal of heterogeneity among lymphocytes both in size and morphology. Most lymphocytes are of a small variety, with a high nuclear to cytoplasmic ratio. Small lymphocytes are divided into T-cells and B-cells. About 65-85% of human T-cells are of the small lymphocyte type. These cells differ by cell surface proteins, which are used to distinguish the two.

T-Lymphocytes (T-cells)

Most human T-cells express three special surface glycoproteins that can be used to differentiate the subtypes. Helper T-cells have different surface markers and assist the main cytotoxic T-cells (also labeled CD8+ T-cells) during immune attacks. T-cells and B-cells also contain receptors for Fc region of an antibody, which are thought to regulate lymphocyte responses. T-cells contain a number of lysosomal hydrolase enzymes, such as acid hydrolases, β-glucoronidase, acid phosphatase, and alpha naphthyl esterase (ANAE), which assist in killing microbial invaders and tumor cells.

T-cells play a major role in controlling both infectious invasions and neoplasms. Specialized T-lymphocytes, called T regulatory cells (T_{reg}) constitute a special type of T-cell that reduces inflammatory responses so as to prevent the immune system from over-reacting and producing excessive damage to surrounding tissues. Its antiinflammatory, immune suppressant activity is connected to the release of high levels of IL-4 and IL-10, the antiinflammatory cytokines. Tumor eradication is blocked when the infiltrated T-cells are predominately T_{reg}-type lymphocytes, which is commonly seen, especially in human malignant gliomas. Newer evidence also suggest that lymphocyte invasion in MS might be neuroprotective by consisting mostly of T_{reg}.

B-Cells

These antibody-producing lymphocytes represent nearly 5–15% of the circulating lymphocytes in the blood. Most circulating peripheral B-cells express both surface IgM and IgD antibodies and a smaller number express IgG, IgA, or IgE antibodies. B-lymphocytes in specific locations, such as mucosal surfaces, are more likely to contain the latter three immunoglobulins. An example of this is the high concentration of IgA antibody-containing B-cells in the gut.

These surface antibodies form specific receptors and they also possess other surface receptors, which nonspecifically bind to antigens. A majority of B-cells also have the major histocompatibility complex-class II (MHC class 2) product HLA-DR, which plays a role in regulation of immune responses. Mature B-cells contain complement receptors such as C3b (CR1) and C3d (CR2).

Once stimulated by specific antigens, these cells differentiate and proliferate into clones of cells with specific roles. The nonspecific cells form effector cells, such as cytotoxic T-lymphocytes and specific lymphocytes from memory cells. When exposed to this same antigen again, these memory cells differentiate into attacking effector cells and a small pool remains as memory cells. These memory cells allow the lymphocytes to react quickly to a subsequent challenge and to remember the invading antigen signature so that reexposure can elicit a more rapid and intense reaction, thus avoiding the need to go through the initial steps of activation all over again. This allows the immune system to suppress the invading organism or tumor before it can reach population numbers that can overwhelm the immune system.

Resting lymphocytes, especially memory cells, circulate through the tissues and lymphoid organs by way of the thoracic duct and blood, thus allowing for immune surveillance, a process essential in preventing cancer cell development as well as invasion of microorganisms. Lymphocyte competence can be tested by exposing them to various mitogens in vitro. For example, T-cells can be activated
when exposed to the mitogens phytohemagglutinin (PHA) and concanavalin A (Con A). Pokeweed mitogen (PWM) stimulates T- and B-cells and lipopolysaccharides (LPS) stimulate a number of immune cells.

Null Cells
Another population of lymphoid cells lack specific markers for either T- or B-cells and are found in the circulation as noncommitted cells. They contain Fc receptors for IgG recognition and have the appearance of a large granular lymphocyte. One of the more interesting cells among the null cells is the natural killer cell (NK cell), which can nonspecifically kill tumor cells and virally infected cells. Another cell within this group is the antibody-dependent cellular cytotoxic cell (ADCC), which also kills nonspecifically, but require an antibody tag on the surface of the target cell. They also play an important role in killing tumor cells.

THE ADAPTIVE IMMUNE SYSTEM IN ACTION; HOW ALL THESE CELLS WORK TOGETHER TO RECOGNIZE A PREVIOUS INVADER ORGANISM

T helper lymphocytes
Unlike other T-cells, helper T-cells have no cytotoxic or phagocytic function, but rather play a complex role in regulating lymphocyte function during specific situations, that is, they help regulate immune reactions. T-helper cells are often referred to as CD4+ T-cells because they express a surface protein CD4. These cells interact with APCs to help regulate immune reactivity utilizing a finely coordinated system of secretory immune factors, such as cytokines [Figure 8]. The activation of these cells is carefully controlled through a series of regulatory processes, beginning with recognition (signal 1). This phase requires interaction with APCs following absorption of the antigen and subsequent movement to a regional lymph node where it presents the antigen signature information to the inactive T-cells’ receptors. This interaction between the APC and the T-cell results in T-cell activation. Memory T-cells record and store this information as well so that when a second exposure the T-cells and T-helper cells do not have to repeat this complex process.

The initial recognition phase and verification of an invader
This initial recognition phase is followed by a verification phase (signal 2), which makes sure the information is correct and an immune reaction is truly needed—a sort of failsafe system. At this stage co-stimulatory molecules interact with these cell combinations and include the surface proteins CD28, CD80, and CD86. Once confirmation has taken place the immune reaction can proceed. Should confirmation be denied, the lymphocytes become anergic permanently.

Interleukin-2 and the stimulation of other T-cells
The cytokine interleukin-2 (IL-2) also stimulates proliferation of other T-cells. T-helper cells are lymphocytes that are used to regulate what type of reaction is needed. The uncommitted T-helper cell is called Th0 helper cells. Based on the particular signature of the stimulating molecule, the Th0 helper cell will transform into a Th1 cell, TH1-7 cell, or a Th2 helper cell [Figure 5]. Some of these cells will further differentiate into regulatory T-cells (Treg), which suppress immunity and inflammation, bringing balance to the system.

TH1, TH2, and TH17 cells in the adaptive immune response
You will hear a lot about the Th1/Th2 reaction, but most immunologists agree that this classification if far too simplistic. Recently, a third T-cell determinant has been discovered, the Th17 cell, which is a proinflammatory type T-cell. Basically, both the Th1/Th17 are primarily cytotoxic, with the generation of a number of proinflammatory cytokines, and is associated with a number of autoimmune disorders [Figure 9]. Th1 is associated with a release of proinflammatory cytokines, such as IL-1ß, TNF, IL-6, and IL-23. Th17 stimulates the release of IL-17, which can induce neutrophil-rich type inflammation and recent evidence indicates that it may be the main T-helper cell type involved in autoimmune disorders.

Until recently, it was thought that Th1 reactions were the main reaction involved in autoimmune disorders, such as experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis. Interestingly, animals in which Th17 has been knocked out do not develop EAE with the usual immune stimulation, whereas knock out of Th1 had no effect on EAE development or progression.

The most important reaction against invading organisms and tumors is via Th1 and Th17 reactions. The Th2 profile is generally considered to be antiinflammatory and immune suppressing, yet still we see important exceptions.

The autoimmune disease asthma, for example, is associated with a predominant Th2 type response.

The Th2 cytokines include the antiinflammatory cytokines IL-4, IL-10, and IL-13. There appears to be a considerable degree of dynamic plasticity within the system and the lines tend to blur. Th2 cytokines can directly kill tumors cells, but most often they assist CD8+ cytotoxic T-cells in doing so. Th2 reactions mainly involve humoral antibodies.

The Th1 system operates mainly through macrophage/microglia and produces a number of cytokines, including IL-2, a primary proinflammatory cytokine. Also released are transforming growth factor-beta (TGF-ß) and IL-10, both important in down-regulating immune reactions so as to prevent immune overreaction. This again, demonstrates the fine-tuning of the immune reaction.
designed to prevent damaging immune excesses. The Th1 system also stimulates the proliferation of cytotoxic CD8+ T-cells, which play a major role in removing virally infected cells and in tumor immunity.

**TH1 cells stimulating interferon-gamma and interleukin 12 in the adaptive immune response in recruiting more specialized lymphocytes**

Another important regulating factor released by Th1 cells is interferon-gamma (IFN-γ), which stimulates the release of IL-12 from dendritic cells and macrophages. IL-12 is known as the T-cell stimulating factor, as it plays a critical role in the production of uncommitted Th0 helper cells and eventually induces the Th1 subtype as well as NK cell proliferation. Its role is to ensure essential immune competence is present. Both IL-12 and IFN-γ also, by negative feedback, suppresses IL-4, an immune suppressor cytokine, so as to maintain essential cytotoxic activity without over-reaction.

**More on the Th2 cells in the adaptive immune response in making antibodies**

The Th2 cells operate primarily through B cells and release a number of cytokines, such as IL-4, IL-5, IL-6, IL-10, IL-13, and TGF-β. These helper cells stimulate B cell proliferation and promote antibody production. Under most conditions, these cytokines reduce the inflammatory response, in particular utilizing TGF-β, but also other factors such as nuclear factor E2-related factor 2 (Nrf2). In addition, IL-10 has been found to be particularly important in preventing immune over-activity, especially in autoimmune diseases. While termination of immune reactions to invading organisms is dependent on apoptosis of activated cells, it is also dependent on the release of these inflammation-suppressing cytokines.

Abnormalities in the balance of proinflammatory and antinflammatory systems are increasingly thought to explain a number of phenomena associated with many neurological diseases, including the neurodegenerative diseases.

**APCs to T-Cells and the adaptive immune system**

These are cells found primarily in lymph nodes, the spleen and the skin and as their name implies, they present the antigen to the naïve (unactivated) lymphoid cells. The typical example given is the Langerhan’s cell in the skin. These cells migrate to a regional lymph node where they interact with a number of T-cells. APCs are dense with class II major histocompatibility complex (class II MHC) antigens, which are critical for the presentation process and for encoding the T-cell with the necessary molecular information for immune reactivity [Figures 10 and 11].

**The dendritic cell and interaction with the b cells; microglia as apcs in the CNS**

A similar cell, called a follicular dendritic cell, interacts with B cells within draining lymph nodes and the spleen to activate antibody production. Microglia in the brain are also APCs.

Actual presentation of the antigen from the invading microorganism or tumor cells occurs with the involvement of dendritic cells, which can produce large amounts of INF-γ. These dendritic cells are specialized and are mostly located where the environment is in contact with the various tissues, such as the skin, mucosa of the gut, respiratory tract, CNS parenchyma, and urinary tract. In essence, these cells represent communication sites between the innate immune system and the adaptive immune system, with the innate immune system being the guardian at the gate.

Dendritic cells constantly sample surrounding tissues looking for evidence of invasion by microorganisms or formation of tumors. To accomplish this requires a series of cell surface PRRs, which include TLRs. This recognition process can involve pieces of the invader’s cell membrane or other cell components, often enough to establish identifiable pathogen signatures. The immature dendritic cells upregulate surface co-stimulatory molecules, such as CD40, CD80, and CD86, and this combination powerfully stimulate T lymphocyte activation.

Once the dendritic cell is stimulated by the pathogen, the cell rapidly produces the cytokine IL-12, which pushes the naïve CD4+ T-helper cell toward a Th1 phenotype, that is, the proinflammatory mode. Dendritic cells communicate with local cells and tissues and receive information at a distance by circulating cytokines, thus keeping them informed of conditions within the body.

**Complement**

Complement proteins can enter and circulate in the blood in an inactive state. Once triggered, they further activate a series of other complement proteins, resulting in complex binding compounds that can kill or assist in killing a variety of invading pathogens or tumor cells. Basically, they function as opsonizing compounds that bind to microbes and promote phagocytosis.

An example of this process involves five activated proteins within a complement cascade that forms a multunit protein referred to as the membrane attack complex (MAC), which can embed itself into the membrane of a microbe or tumor cell thereby forming transmembrane channels. This disrupts the integrity of the invading cell, leading to its death.

Complement also plays an important role in inflammatory mechanisms, vasodilation, microvessel permeability to proteins, and chemotaxis.

In the classical complement pathway (called this because
Figure 8: Basic process of T-lymphocyte activation by exposure
to an APC and IFN-γ. A number of cytokines are involved in the
activation process.

Figure 9: Demonstration of the classification of Th1 and Th2 helper
T-cells and their functions in controlling immune reactivity.

Figure 10: Major Histocompatibility Complex Class I [MHC
class I] is a surface receptor that plays a vital role in immune cell
responses and plays a central role in self-identification. By binding
peptides derived from the cytosol and presenting them to CD8+
lymphocytes, the MHC class I allows the lymphocytes to determine
if the peptide is of self and to be ignored or foreign and to be attacked.

Figure 11: Major Histocompatibility Complex Class II [MHC
class II]. When immune cells, such as macrophages and microglia [APCs]
are activated, MHC class II complexes with invading extracellular
peptides and these in turn interact with lymphocytes, such as the
CD4+ T-cell.

Figure 12: Demonstration of the interaction between macrophages
and cytokines, which can trigger a switching from a classical
cytotoxic active macrophage (M1) to the alternative pathway,
producing an immune suppressant macrophage phenotype (M2).

It was named first), antibodies are required to activate
the first of the complement proteins (C1q) to start off
the cascade. This exists as an inactive compound called
zymogen, which is activated as a proteolytic enzyme. This
is turn activates the next complement in line and with
each activation there is a tremendous amplification of next
enzyme created. To start the process, the C1q binds to the
Fc portion (a receptor deep in the antibody) of an antibody
that is already bound to the surface of the bacteria.

During nonspecific inflammation, not requiring lymphocytes,
C1q is bypassed and other complement molecules further
down the chain (C3) interact with carbohydrates on the
surface of the microbe or tumor leading to the formation
of C3b, the opsonin complement, which facilitates binding
of the pathogen to phagocytes. This assists the phagocyte in
engulfing the microbe. We call this nonspecific system the
alternate complement pathway.
ALTERNATIVE ACTIVATION PATHWAYS

Critical to successful immune defense is having mechanisms to provide ongoing modulation of the immune attack so as to prevent excessive tissue damage and to eventually terminate the attack and initiate tissue repair, once the danger is over. We call this alternative activation as opposed to classical activation [Figure 12].

The immune system has a number of modulating mechanisms previously discussed, which are finely coordinated during immune activation.

One of the most common mechanisms entails ongoing trafficking of immune receptors to and from the immune cell surface membrane, regulated by immune mediators. This changes the sensitivity of the immune cell to the antigen, thus preventing overreaction.

Another modulator system entails the adaptor proteins within the cytoplasm, such as MyD88, which is connected to all TLRs. More recently, micro RNA (noncoding RNA) has been found to regulate inflammation by regulating NFκB signaling, a central transcription regulator of inflammation.

The immune system also has an elaborate system of decoy receptors, for example, the soluble IL-1 receptor decoy (IL-1RD), which are expressed by macrophages. By binding with extracellular IL-1, the decoy prevents binding of the cytokine with the active IL-1 receptor on the immune cell.

Interestingly, one sees elevated IL-1RD in the CSF of Alzheimer’s patients, which may prevent phagocytosis of neurotoxic Aβ. Other decoys are found for IL-6 and TNF-α. IL-1 also has a suppressor counterpart, IL-1ra, which suppresses IL-1 activity directly.

Classical activation actually produces both proinflammatory and antiinflammatory cytokines, with a predominance of proinflammatory mediators. It should be appreciated that this is a dynamic process and the balance can change during the inflammatory process. With a Th2 shift, one sees an elevation in the antiinflammatory cytokines, such as IL-4, IL-13, TGFβ, and IL-10. These can be secreted from a variety of immune cells, but in the brain the major sources are microglia and macrophages.

Alternative activation of macrophages (aaMAC) involves IL-4 acting through the cell signaling molecule STAT6, which in turn increases the release primarily of IL-10 and TGFβ. IL-10 depends on STAT3 for its antiinflammatory, immunosuppressive activity. Macrophages that phagocytize apoptotic cells assume an immunosuppressive phenotype called M2. These antiinflammatory cytokines also suppress MHC class II and the co-stimulatory proteins and thereby interfere with antigen presentation, thus proinflammatory, active macrophages (M1) are suppressed by this switching process.

In addition to a suppression of immunity, M2 macrophages also express protective elements and growth factors, such as nerve growth factor (NGF), Bcl-2, Bcl-xl, and insulin-like growth factor-1 (IGF-1). This aids in repairing damage done during the immune attack to surrounding neural elements and neurons, something labeled bystander injury. TFGβ also suppresses further migration of systemic immune cells into the CNS. In addition, microglia guide neural stem cells to the site of inflammation. The M2 cell, by promoting phagocytosis, aids in removal of cellular debris, which further reduces microglial immune stimulation and excitotoxicity.

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