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Basic Principles in Immunology

Relevance for Studies in Psychoneuroimmunology

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Introduction

Over the past century our knowledge of the immune system and how it functions has grown exponentially. This is especially true in regard to how it relates to and interacts with various physiological systems, including the central nervous system. An important focus of the field of neuroimmunology is to elucidate the ways that the immune system influences neuronal function and subsequently, behavior and cognition through the modulation of cytokines and hormones, especially stress hormones such as corticosteroids. Since the intimate relationship between the immune system and brain function has come to light, research in this field has broadened into psychoneuroimmunology, which specifically addresses the role of the immune system in the development of psychiatric disorders, including depression and anxiety. The purpose of this section is to provide a general overview of basic immune function, describing both the components of the immune system and the various modes of immunity employed in an immune response. Additionally, we will explore the validity of some of the most widely used methods and models for psychoneuroimmunology applied to the study of interactions between immunological processes and behavior and cognition as they relate to mental disorders in humans.

The Components of the Immune System

To understand how the immune system influences the brain, and subsequently, behavior and cognition, it is vital to understand how the immune system functions. The immune system
comprises two major components: specialized cells that carry out the various functions of the immune process, and the chemical messengers that allow these cells to communicate, not only with each other, but with other cells and tissues within the body. These partners in immune function must perform a precise and complex dance in order to maintain homeostasis and, when necessary, to mediate an inflammatory response. In general, as part of the inflammatory response damaged or infected cells secrete chemical messengers called chemokines that serve to attract specific immune cells, which in turn release various cytokines that influence the types of cells and modes of immunity that will be employed to eliminate any potential pathogens. Once these threats have been neutralized the process continues, as immune cells and their chemical messengers also function to mediate tissue repair and regeneration. A lack of coordination from either partner can result in deleterious consequences, including the development of allergies, as well as autoimmune and immune-deficiency disorders.

Cytokines and chemokines – the immune system’s messengers

Cytokines and chemokines are protein and glycoprotein molecules synthesized and secreted by cells as part of the immune response. Chemokines are a specialized class of cytokines that derive their name from their role in chemotaxis; a majority of these soluble factors are chemoattractants that serve to guide immune cells to the site of infection. They are characterized by their small size and the presence of four cysteine residues (named C) which contribute to their tertiary structure. They are divided into four families (C, CC, CXC and CX3C) based upon the location of the first two C residues. Chemokines in the C group differ from the other chemokine families in that they contain only two cysteines; secretion of these chemokines attracts T-cell progenitors to the thymus. The CC chemokines have two adjacent cysteines near the amino terminus, while the relevant cysteines in the CXC chemokines can be found at the N-terminus separated by a single amino acid (X). Similarly, the CX3C chemokines have three intervening amino acids; thus far, fractalkine is the only chemokine with this structure that has been identified. In addition to their role in immune function, chemokines contribute to a variety of biological functions, especially in the brain, as will be discussed later.

The major cytokines consist of interleukins (IL), interferons (IFN) and colony-stimulating factors, as well as various growth factors and eicosanoids, including prostaglandins. Cytokines are mainly produced by immune cells and also by a variety of other cell types including brain cells. The specificity of the elicited immune response is dictated by the expression of cytokine receptors that are widely expressed in tissues and organs. Additionally, some cytokine receptors exist in a soluble form and can act as inhibitors of cytokine activity through competitive binding of their ligands. To differentiate between cytokines’ biological activity they are often described as either pro-inflammatory or anti-inflammatory; upon damage to or infection of cells and tissues, pro-inflammatory cytokines are produced and secreted to stimulate immune system activation. The induction of cytokine expression tends to occur in a step-wise manner, with the expression of certain cytokines dependent upon the prior expression of others; for example, IL-1 is necessary to induce the production of IL-2, IL-6 and tumor necrosis factor (TNF). Anti-inflammatory cytokines, such as IL-10, are also released during inflammation in order to dampen and eventually terminate pro-inflammatory cytokine activity. In many instances, the cytokines IL-4 and IL-13 are referred as anti-inflammatory because they oppose the effects of inflammatory cytokines IL-2 and IFN-γ. However, many inflammatory processes such as allergic inflammation are mediated by the actions of IL-4 and IL-13. Thus, describing
cytokines purely by their pro- or anti-inflammatory properties can be misleading since they are pleiotropic in nature and are involved in many biological processes. Maintaining a balance in cytokine and chemokine signaling is vital for sustaining immune homeostasis and stimulating the appropriate immune cells as part of the immune response.

The cells of the immune system

The circulatory system serves as the main highway for the cells of the immune system, so it is not surprising that immune cells are derived from the same source as the other major components of blood. During prenatal development the spleen and liver are responsible for producing both red blood cells and white blood cells; however, once the skeleton begins to develop and the bone marrow becomes established, this responsibility shifts to hematopoietic stem cells (HSCs) within the bone marrow. HSCs give rise to the three cell lineages of the blood and immune system: the erythroid lineage, the myeloid lineage, and the lymphoid lineage (see Figure 1.1). Currently, the general consensus for how these lineages arise is that the initial progeny of HSCs are multipotent progenitor cells (MPPs) which in turn give rise to common myeloid progenitor cells (CMPs). Progeny of these CMPs maintain expression of myeloid specific genes, but can undergo further restriction into either erythroid or lymphoid progenitors. Thus, the myeloid lineage may be considered the default fate for CMPs unless directed towards either erythroid or lymphoid lineages through changes within the milieu of the stem cell niche, including alterations in cytokine expression. Ultimately, the erythroid lineage will develop into red blood cells and platelets while the myeloid and lymphoid lineages will give rise to the cells of the immune system.

The myeloid lineage

Members of the myeloid lineage include monocytes, granulocytes and mast cells. The primary function of monocytes is to migrate out of the vasculature and into tissues where they mature into macrophages that will monitor the body and destroy potential pathogens through phagocytosis. Macrophages can be further classified into mobile or fixed macrophages. The alveolar macrophages of the lungs and the dendritic cells of the epidermis are examples of mobile macrophages that can freely travel within the interstitial space, whereas the Kupffer cells of the liver remain fixed in place.

The granulocytes, named for the multiple granules found within their cells, comprise three types of cells: neutrophils, eosinophils, and basophils. These polymorphonuclear cells (PMNs) are confined primarily to the blood stream until activation by cytokines and chemokines released by damaged cells and tissues. These messengers prompt the PMNs to migrate into the interstitial space where they will hunt down and destroy invading pathogens. Neutrophils, which make up the greatest proportion of PMNs, are phagocytic cells that are among the first cells recruited to eliminate invading pathogens. In addition to destroying foreign cells by phagocytosis, neutrophils can also degranulate and release anti-microbial chemicals such as gelatinase and cathepsin. Interestingly, neutrophils have also been observed extruding filaments of DNA and associated proteins that can act as nets to entrap microbes; these extracellular structures provide an alternate method of destroying pathogens and may prevent their spread into the surrounding tissue. Lastly, neutrophils also release cytokines and thus can enhance the inflammatory response by recruiting more immune cells to the site of infection. The other two types of granulocytes, the eosinophils and basophils, make up a relatively small proportion of the total leukocyte population, but are vital in mitigating the effects of pathogens, especially...
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Figure 1.1  Cells of the immune system. Hematopoietic stem cells (HSCs) within the bone marrow are relatively quiescent stem cells whose progeny, multipotent progenitor cells (MPPs), can differentiate into both erythrocytes and leukocytes. There are three potential lineage fates: the erythroid lineage, which gives rise to both red blood cells (RBCs) and platelets, and the myeloid and the lymphoid lineages, which produce the cells of the immune system. Myeloid progenitor cells differentiate within the bone marrow to produce monocytes, granulocytes and mast cells, which then migrate to their target environments within the blood and tissue. Lymphoid progenitors can also be found within the bone marrow, however these cells will differentiate into precursor B-cells that will then migrate into the lymphatic tissues and organs. In contrast, T-cell progenitors leave the bone marrow and migrate directly to the thymus where they will undergo further proliferation and selection for immunocompetency.

for their role in mediating the innate and adaptive immune responses. Although eosinophils and basophils are perhaps best characterized for their anti-parasitical activities, in recent years their role in tissue and immune homeostasis has been further clarified. As part of the adaptive immune response, eosinophils are rapidly recruited to the site of infection by T-helper 2 (T\textsubscript{H}2) cells, where they release cytokines and lipid mediators, such as prostaglandin 2, as well as cytotoxic chemicals that can destroy invading pathogens. Additionally, they have the capability of acting as antigen-presenting cells to activate both naïve and memory T-cells. Finally, both eosinophils and basophils have also been implicated in the development of hypersensitivity and allergies, perhaps due to their relationship with T\textsubscript{H}2 cells and mast cells.

Mast cells are functionally and morphologically similar to eosinophils and basophils; they play a vital role in the immunity against parasites, and facilitate tissue repair by stimulating angiogenesis, the growth of new blood vessels. However, these myeloid cells are found mainly within tissues adjacent to the external environment, especially within the mucosae of the respiratory and gastrointestinal tracts, and are perhaps best-known for their role in allergic responses. Mast cells are also found in the brain, particularly in some nuclei of the thalamus.
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The granules within mast cells store a variety of cytokines and chemokines that facilitate the inflammatory process, as well as histamine which not only dilates blood vessels and is responsible for the pain and itchiness associated with an allergic reaction, but which can also act as a neurotransmitter. Another neurotransmitter, serotonin, has also been found within mast cells, and although the role of these neurotransmitters is not yet clear, they may be involved in cross talk between the immune system and neurons, especially those of the enteric nervous system. Interestingly, in addition to direct damage or the binding of antigens, degranulation of mast cells can also be initiated by various neuropeptides, further supporting the possibility that mast cells represent a link between the immune system and the nervous system.

The lymphoid lineage

Lymphocytes derive their name from the fact that they reside primarily within the tissues of the lymphatic system. These tissues include a network of reticular fibers that can be found in virtually every organ of the body; these fibers converge upon the lymph nodes and the two major organs of the lymphatic system: the spleen and the thymus. The main function of the lymph nodes is to filter out and clear lymph as it travels along the lymphatic vessels. Resident macrophages remove and destroy any microbes or cellular debris while lymphocytes monitor the lymphatic stream for the presence of foreign antigens. The lymphocytes include B-lymphocytes, T-lymphocytes and natural killer (NK) cells.

B-cells differentiate within the bone marrow and migrate into the lymph nodes and spleen. Here they will remain in a precursor stage until activated by an antigen, at which time they will undergo rapid proliferation and maturation into antibody-secreting plasma cells. Membrane-bound immunoglobulins (Ig), including IgM and IgD, on the surface of precursor B-cells act as receptors for intact antigens. The binding of the antigen stimulates the production of secretory immunoglobulins, usually referred to as antibodies, including IgM, IgG, IgA and IgE. These antibodies consist of a conserved region and a variable region. It is the conformation of the variable region (the product of the genetic recombination of several genes within the immunoglobulin super-gene family) that makes the antibodies specific for their target antigen. Interestingly, lymphocytes are the only somatic cells that rearrange DNA to produce new protein variants as part of their phenotype.

Once antibodies have been secreted into the extracellular space they can facilitate the removal of pathogens in a variety of ways. By binding to antigens on the surface of pathogens they can make the pathogen more visible to macrophages. That is, the antibody serves as an opsonin (from the Latin “to relish”), that marks the pathogen as a target for phagocytosis by macrophages; this will be facilitated by the Fc region of the antibody molecule binding to Fc receptors on the macrophage. Additionally, some immunoglobulins are capable of binding to and activating other effector cells, including granulocytes and mast cells. In the case of IgG, binding to platelets allows for the transfer of immunity across the placenta, which is vital for the development of the fetal immune system. The binding of the Fc region of IgE to the Fc receptor on mast cells results in mast cell degranulation and release of inflammatory mediators such as histamine. The production of IgE antibodies against harmless compounds such as pollen or albumin is responsible for the establishment of allergies.

Although the role of different antibodies in the immune response is quite varied, their primary function is to facilitate the removal of pathogens; however, in order to do so, they must be able to bind to antigens. Individual immunoglobulins are specific for only one or two closely related antigens, though they may be able to bind to other related antigens with lower affinity. However, the immune system cannot sustain an army of B-cells for every possible antigen that the body may encounter. Instead, precursor B-cells expressing a specific antibody
monitor the spleen, lymph nodes, and other peripheral lymphatic organs for the antigen that matches its antibody, much like pairing up two pieces of a puzzle. Upon successful binding of the antibody and antigen, the B-cell will undergo a period of rapid proliferation, or clonal expansion, making multiple replicas that will then mature into antibody-secreting plasma cells. Additionally, a subset of B-cells will become memory B-cells, which can rapidly mature into plasma cells should they encounter their specific antigen again.

Unlike the B-cells, T-cells do not differentiate in the bone marrow. Progenitors of T-cells instead migrate to the thymus where they continue to proliferate and undergo thymic selection, a process that ensures that T-cells are immunocompetent. Although initially considered a minor player in the immune response, the importance of T-cells in maintaining immune homeostasis and in modulating the immune response has become abundantly clear. The establishment of their critical role in autoimmune diseases and allergies, as well as immune-deficiency disorders such as HIV/AIDS, has been a major advance in immunology. A naïve T-cell (T\textsubscript{0}) has the potential to differentiate into a variety of effector T-cells, which can be distinguished by the expression of recognition proteins known as cluster of differentiation (CD) proteins. Helper T-cells (T\textsubscript{H}), also referred to as CD4\textsuperscript{+} T-cells, modulate both the innate and adaptive immune response; among their many functions, they assist in the maturation of B-cells into plasma cells and memory B-cells, and activate cytotoxic T-cells as well as macrophages. Cytotoxic T-cells (T\textsubscript{C}), which are CD8\textsuperscript{+}, attack and destroy virally infected cells as well as tumor cells. The major role of regulatory T-cells (CD4\textsuperscript{+}, CD25\textsuperscript{+}, FoxP3\textsuperscript{+}, T\textsubscript{reg}), sometimes referred to as suppressor T-cells, is to shut down T-cell mediated immune responses. Finally, memory T-cells are antigen-specific subsets of CD4\textsuperscript{+} and CD8\textsuperscript{+} T-cells which have been previously activated and have the capacity to remain viable for long periods of time. Upon re-exposure to the antigen they will rapidly proliferate and activate both T\textsubscript{H} and T\textsubscript{C} cells so that the immune system can specifically target and destroy the invading pathogen.

The final class of lymphocytes is the natural killer (NK) cells; these cells take part in the innate immune response and act primarily against cells infected by viruses or rogue cells that have become cancerous. Unlike phagocytes, NK cells destroy their targets through the release of perforins, cytolytic enzymes that punch holes in the membrane of the targeted cell. In addition, NK cells discharge a class of proteases called granzymes, which enter the perforated cell and catalyze cell death via apoptosis. Since many of the cells that NK cells target are infected by viruses it is vital that the destruction of the cell is contained; if the cell was merely lysed any viruses that had succeeded in reproducing would be released to infect other cells.

In order to maintain immune and tissue homeostasis myeloid and lymphoid cells work together in a precise and coordinated dance choreographed by cytokines and chemokines. Although each partner is responsible for specific facets of an immune response, they are also dependent upon each other in order to provide the best protection for the host. So as to provide an optimal defense against potential pathogens these components of the immune system employ diverse strategies for identifying and eliminating various microbes.

**Modes of Immunity**

The immune system must be able to cope with a variety of potential pathogens, as well as tumor cells and other damaged host cells, while mitigating possible damage to healthy cells and tissue. This requires the correct identification of potentially deleterious microbes and cells followed by their targeted elimination. In order to accomplish this the immune system
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utilizes two distinct, though interdependent, forms of protection which work together in a complex yet highly coordinated assault on pathogens that attempt to invade the body. The innate immune system is a general, non-specific form of defense comprised of anatomical barriers, which serve as a blockade against a majority of microorganisms, and immune cells, including granulocytes, mast cells, macrophages and NK cells, which can recognize and attempt to destroy potential pathogens that breach the barricades of the skin and mucosae. The function of the innate immune system is modulated and enhanced by the adaptive immune system, a specific form of defense which targets and marks pathogens for elimination. However, unlike the innate immune system, a hallmark of the adaptive immune system is that it displays memory, a trait that has been exploited in the development of vaccines. The adaptive immune system evolves in response to the pathogens it encounters over the lifetime of the host, selecting and maintaining a pool of memory B-cells and T-cells specific for antigens the body has been exposed to, so that should the body be invaded by the same pathogen in the future, it can rapidly and specifically target it for destruction.

In order to convey how the immune system contends with the variety of extracellular and intracellular pathogens the host may encounter, the type of defense utilized by immune cells is often described as either cell-mediated immunity or humoral immunity. Cell-mediated immunity is typically modulated by helper T-cell class 1 (T\textsubscript{H}1) cells, which orchestrate attacks against intracellular bacteria and viruses, as well as tumor cells. This is often through the release of cytokines, such as interferon, that catalyze the programmed cell-death pathways of infected cells; this process results in the elimination of the pathogen and limits its ability to spread to other cells. In contrast, humoral immunity is targeted against extracellular pathogens, including bacteria, fungi, and helminthes; this form of immunity is mediated by T\textsubscript{H}2 and, to a lesser degree, T\textsubscript{H}17 cells, which enlist granulocytes and mast cells to facilitate the destruction of these pathogens. Additionally, T\textsubscript{H}2 cells can stimulate the release of antibodies from B-cells; these antibodies can then bind to pathogens and mark them for destruction by macrophages.

To function correctly, the immune system must be able to distinguish between healthy host cells and potential pathogens, as well as damaged host cells, including infected or cancerous cells, in order to identify which ones must be destroyed. Members of the innate immune system utilize a set of pattern-recognition receptors (PRRs) that recognize highly conserved motifs that are unique to non-mammalian cells, including components of the bacterial cell wall, such as lipopolysaccharide (LPS) and peptidoglycan, as well as viral nucleic acid structures, such as single-stranded (s.s.) and double-stranded (d.s.) RNA. These molecular structures are referred to as pathogen-associated microbial patterns, or PAMPs; the binding of a PAMP to its receptor triggers signaling pathways that activate transcription factors, such as NF-κB, and induce the expression and secretion of pro-inflammatory cytokines and chemokines. This process initiates an inflammatory response that involves both the innate and adaptive immune systems, with the ultimate goal of eliminating the invading pathogens.

As shown in Figure 1.2, there are four main families of PRRs: Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-1-like receptors (RLRs) and C-type lectin receptors (CLRs). Of these, the TLRs are the best characterized; however, ongoing research into the other three classes of PRRs, as well as other lesser-known receptors, indicates that the innate immune system, although non-specific, is efficient at identifying potential pathogens. TLRs are transmembrane proteins with a leucine-rich extracellular domain and a conserved region, the Toll/IL-1 receptor domain, on the cytoplasmic tail. These receptors are expressed in tissues involved in immune function, including the spleen and leukocytes, and on cells within the lungs and gastrointestinal tract, as well as other environments that are exposed to the external environment.
Figure 1.2 The innate immune system. A) Activation of the inflammatory response requires the recognition of highly conserved non-mammalian motifs referred to as pathogen associated molecular proteins (PAMPs). These include bacterial cell wall components such as lipopolysaccharide (LPS) and peptidoglycan (PGP), as well as the protein flagellin, viral nucleic acids (both s.s. and d.s. RNA) and fungal cell-wall components. B) These PAMPs can be identified by a wide assortment of pattern recognition receptors (PRRs) found on a variety of cells, especially those of the innate immune system. The lower panel illustrates how activation of PRRs within the cell by various pathogens initiates signaling pathways that culminate in the production of pro-inflammatory cytokines which will ultimately serve to stimulate the adaptive immune response.
Remarkably, TLRs are also expressed in the brain. Members of the TLR family are found on both the cell surface and within intracellular compartments; this facilitates the detection of pathogens within the extracellular space as well as those that are able to penetrate the cell. The ability of TLRs to recognize a variety of PAMPs is further enhanced by the fact that many exist as heterodimers and/or form associations with various adaptor and accessory molecules that influence their specificity for numerous substrates. TLRs are capable of recognizing all classes of microorganisms, including bacteria, fungi and parasites, as well as viruses. The other three classes of PRRs have a narrower repertoire of PAMPs that they can identify. Recognition of fungal invasion is mediated by CLRs, another transmembrane PRR; interestingly, these receptors bind to carbohydrate structures found in fungi, such as α-glucan and mannan, in a Ca$^{2+}$-dependent manner. Intracellular PRRs include NLRs and RLRs, which serve to detect pathogens that have penetrated the cell membrane; these PRRs provide a layer of defense in cells that typically do not express TLRs, such as the epithelial cells that line the gastrointestinal tract. NLRs are activated upon infiltration of the cell by bacterial PAMPs, as well as damage-associated molecular patterns (DAMPs) which arise after the integrity of the cell has been compromised. Infection of cells by viruses activate RLRs, which react to the detection of double-stranded RNA; these RNA helicases are vital for anti-viral responses, including the release of IFN-β, which will not only lead to the destruction of infected cells, but will also serve to activate the T$\text{H}$1 cells as part of the adaptive immune response.

In contrast to cells that utilize PRRs for the recognition of general motifs expressed by a variety of different pathogens, cells of the adaptive immune system must be able to identify and specifically target pathogens via each pathogen’s particular antigens. For example, infection of a cell by influenza will stimulate the innate immune response through the activation of RLRs; however, the optimal immune response would be to destroy the virus before it infected host cells. Here the virus has an advantage because the primary proteins on its surface, hemaglutinin and neuraminidase, are constantly evolving and thus cannot act as PAMPs because different strains express diverse proteins. Nevertheless, they can serve as antigens, unique identifiers of an infection by a specific strain of influenza, and therefore initiate the adaptive immune response.

Nevertheless, any protein or cellular component can potentially act as an antigen, including those on host cells. This requires that lymphocytes be able to distinguish between “self” and “non-self” in order to avoid an autoimmune response. Essentially all cells found within the body express the major histocompatibility complex (MHC) proteins, which act as identifiers of “self”; like fingerprints, MHC molecules are unique to each individual and consequently play a major role in affecting compatibility between donors and recipients of transplanted blood and organs. Additionally, MHC molecules function to alert the immune system of an invasion through the presentation of antigens. Members of the MHC gene family encode two different classes of MHC molecules. Class I MHC proteins are found on virtually all cells and function primarily in “self” recognition, whereas members of Class II MHC proteins are expressed on specialized antigen-presenting cells (APCs); of particular note are the dendritic cells (DCs), which play a vital role in activating T-lymphocytes, and B-lymphocytes, which generate antibodies and target invading cells for destruction.

The stimulation of naïve T-cells by DCs is vital for mounting an offense against invading pathogens (see Figure 1.3). Activation of the T-cell receptor (TCR) requires binding of both an antigen and the MHC, which initiates the maturation of the naïve T-cell; however, polarization (fate specification into T$\text{H}$1, T$\text{H}$2, etc.) is dependent upon the milieu of cytokines present in the microenvironment as well as the interaction between the antigen and the T-cell. The first class
In addition to destroying pathogens by phagocytosis, various macrophages and dendritic cells process the pathogen for antigen presentation in order to activate an adaptive immune response. As seen in the upper left corner, the intact antigen is bound to an MHC II molecule on the antigen-presenting cell (APC); this acts as a ligand for the T-cell receptor (TCR) and CD protein on the surface of a naïve T-cell (T$_0$). Polarization of the T$_0$ cell depends upon the presence of both the antigen and cytokines. In the presence of IL-12 and IFN-$\gamma$ cells will assume a T$_{H1}$ fate and in turn secrete more IFN-$\gamma$ as well as TNF-$\alpha$, which will then modulate both cytotoxic T-cells (T$_{C1}$) and various cells of the innate immune system. These cells will stimulate the elimination of pathogens through cell-mediated (i.e. intrinsic) processes, such as programmed cell death. On the other hand, if IL-4 is present, T$_0$ cells will differentiate into T$_{H2}$ cells, which modulate the humoral immune response through the activation of assorted innate immune cells. The removal of extracellular pathogens is accomplished by a variety of mechanisms including the degranulation of granulocytes and antibody mediated processes. T$_{H1}$ and T$_{H2}$ mediated immune responses are downregulated by IL-10 and TGF-$\beta$, both of which are secreted by regulatory T-cells (T$_{reg}$); this action is vital for maintaining immune homeostasis.

Of T-cells to be activated is the T$_{H1}$ cells, which will in turn modulate the activity of other T$_{C1}$ cells and B-cells. Various classes of T$_{H}$ cells specialize in specific forms of pathogens and modes of immunity. Among these classes, the T$_{H1}$ and T$_{H2}$ cells are the most renowned, however, other T$_{H}$ cells, including T$_{H17}$, follicular T$_{H}$ (T$_{FH}$) and inducible regulatory T$_{H}$ (iT$_{reg}$) cells, have all recently gained more scrutiny as their roles in the immune response have been further elucidated. The polarization of T$_{H1}$ cells occurs in response to IL-12 and IFN-$\gamma$, some of which is produced by other mature T$_{H1}$ cells. T$_{H1}$ cells are characterized by the release of IFN-$\gamma$ and their role in cell-mediated immunity. Interferons are generally produced in response to viral infections; although an infected cell is not able to protect itself from a virus, it can release interferon which will alert other cells to the presence of the virus and initiate anti-viral measures in those cells, limiting the ability of the infection to spread. Through their release of IFN-$\gamma$, 

Figure 1.3 The adaptive immune system. In addition to destroying pathogens by phagocytosis, various macrophages and dendritic cells process the pathogen for antigen presentation in order to activate an adaptive immune response. As seen in the upper left corner, the intact antigen is bound to an MHC II molecule on the antigen-presenting cell (APC); this acts as a ligand for the T-cell receptor (TCR) and CD protein on the surface of a naïve T-cell (T$_0$). Polarization of the T$_0$ cell depends upon the presence of both the antigen and cytokines. In the presence of IL-12 and IFN-$\gamma$ cells will assume a T$_{H1}$ fate and in turn secrete more IFN-$\gamma$ as well as TNF-$\alpha$, which will then modulate both cytotoxic T-cells (T$_{C1}$) and various cells of the innate immune system. These cells will stimulate the elimination of pathogens through cell-mediated (i.e. intrinsic) processes, such as programmed cell death. On the other hand, if IL-4 is present, T$_0$ cells will differentiate into T$_{H2}$ cells, which modulate the humoral immune response through the activation of assorted innate immune cells. The removal of extracellular pathogens is accomplished by a variety of mechanisms including the degranulation of granulocytes and antibody mediated processes. T$_{H1}$ and T$_{H2}$ mediated immune responses are downregulated by IL-10 and TGF-$\beta$, both of which are secreted by regulatory T-cells (T$_{reg}$); this action is vital for maintaining immune homeostasis.
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T<sub>H</sub>1 cells will also activate T<sub>C</sub> and NK cells, thus enhancing an anti-viral immune response. On the other hand, T<sub>H</sub>2 and T<sub>H</sub>17 cells play vital roles in humoral immunity. T<sub>H</sub>2 cells, polarized by the presence of IL-4, specialize in protection against extracellular parasites such as helminths. Similarly to T<sub>H</sub>1 cells, T<sub>H</sub>17 cells differentiate in response to pro-inflammatory cytokines, including IL-6, IL-21 and IL-23 in combination with transforming growth factor β (TGF-β); these T<sub>H</sub> cells aid in the clearance of extracellular bacteria and fungi. The activity of these cells must be tightly regulated, as overstimulation of either T<sub>H</sub>1 or T<sub>H</sub>17 cells has been shown to elicit autoimmune effects while excessive T<sub>H</sub>2 activity has been implicated in the development of allergies and asthma. Subsequently, regulatory, or suppressor, T-cells, as well as iT<sub>regs</sub> are necessary to dampen and ultimately, terminate the activity of these T<sub>H</sub> cells. Finally, T<sub>FH</sub> cells modulate the maturation of B-lymphocytes into antibody-releasing plasma cells, the second arm of the adaptive immune response.

The Immune System and the Brain

Unlike the peripheral tissues of the body, typical immune cells, such as macrophages, are not commonly found within the brain unless the blood–brain barrier has been compromised. Nevertheless, the brain does have specialized cells with immune-like functions; chief among these are microglia, which are functionally similar to macrophages located in the periphery. Their branching processes make contact with neurons and astrocytes, as well as the endothelial cells of the vasculature. Upon activation, microglia retract their processes and can migrate to sites of injury, where they eliminate apoptotic and necrotic cells through phagocytosis. This function is vital for maintaining homeostasis within the brain. Additionally, both cytokines and chemokines are found throughout the brain, and it has even been suggested that chemokines may act as neurotransmitters or neuromodulators in a variety of brain functions (Rostene <i>et al.</i>, 2011; Tonelli, Postolache, and Sternberg, 2005). Chemokines and their receptors are constitutively expressed in regions associated with adult neurogenesis, including the olfactory bulb and hippocampus, where they may modulate cell proliferation and differentiation (Turbic, Leong, and Turnley, 2011). The only member of the CX3C family, fractalkine, is widely expressed in neurons throughout the brain. The role of chemokines in synaptic transmission has gained greater scrutiny as well, as they have been shown to enhance GABAergic function in various brain regions, including the hippocampus (Bhattacharyya <i>et al.</i>, 2008) and dorsal raphe nuclei (Heinisich and Kirby, 2009, 2010) and may regulate adenosine receptor activity, subsequently inhibiting glutamatergic neurotransmission (Piccinin <i>et al.</i>, 2010). Additionally, astrocytes and microglia secrete both pro- and anti-inflammatory cytokines and, along with neurons, express cytokine receptors, suggesting that even though the brain is sequestered from the peripheral immune system they still communicate with each other.

Over the past few decades it has become abundantly clear that this communication is necessary for the normal functioning of the brain as well as for regulation of the stress response through activation of the hypothalamic–pituitary–adrenal (HPA) axis during times of severe stress and illness (for an exhaustive review please see Yirmiya and Goshen, 2011). The role of T-cells in the modulation of brain function is just beginning to be elucidated; although T-cells are not typically found within the brain parenchyma they do migrate to the meninges and can be found within the blood vessels of highly vasculated regions such as the hippocampus (Brynskikh <i>et al.</i>, 2008). Interestingly, although the anti-inflammatory cytokine IL-4 is most
often associated with beneficial effects on cognitive function, pro-inflammatory cytokines IL-1, IL-6 and TNF-α have also been linked to maintaining proper brain functioning. These effects are elicited through a variety of mechanisms, not least of which is the secretion of neurotrophic factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF) from both brain cells and immune cells. However, excessive release of these cytokines, often as a consequence of severe stress or an inflammatory response, is detrimental and has been linked to impairments in learning and memory, as well as neural plasticity and neurogenesis. Mast cells are also found in the brain and shown to have important modulatory functions including modulating sexual function and emotional states (Nautiyal et al., 2008). These are just a few examples of the importance that immune cells, immune genes, neural-to-immune and immune-to-neural signaling have on brain function and behavior. Due to the relationship between certain inflammatory processes with a variety of cognitive and mental disorders, including depression, schizophrenia and autism, the role of the immune system and immunity on the brain has come under greater scrutiny. Thus, several models have been developed to study the impact of immune system activation on cognition and behavior.

**Methods in Immunology with Relevance for Psychoneuroimmunology**

The research methodology employed in the study of immune system function is extensive and covers all the aspects of biomedical research, from molecular and cellular to animal models and clinical studies. The present section will focus on some basic and validated methodologies developed in the field of immunology that have relevance for the study of emotional and cognitive effects of immune activation. Most of the experimental models involve the use of whole animals because the study usually demands testing in a behavioral setting aimed at evaluating emotional and cognitive functions. Some of these models have also been employed in human studies, with appropriate modifications for compliance with clinical studies. Furthermore, some in vitro cell culture models have also been employed to study specific interactions between immune cells and neurotransmitter systems known to modulate cognition and emotion. Two main methods have been widely used in psychoneuroimmunology to elicit an immune response that may be reflected in changes in behavior and/or mental and cognitive functions. One is using live infectious agents such as viruses, bacteria and parasites that have limited lethality or are capable of establishing permanent infections without resulting in death of the host. Some examples are the use of influenza viruses, neurotropic retroviruses and Toxoplasma gondii (T. gondii) in schizophrenia research, and the use of attenuated strains of bacteria in depression research. The second method is probably the most often employed: it consists of using molecules that mimic the presence of live infectious agents and therefore elicit an immune response similar to the pathogen. Some examples are the use of polysaccharides such as lipopolysaccharides from Gram-negative bacteria, polyriboinosinic-polyribocytidilic acid (poly I:C) mimicking viral nucleic acid, and proteins with particular antigenic properties such as albumin from chicken egg or myelin basic protein. These models have been complemented by behavioral pharmacological studies using direct administration of cytokines in the brain. The following sections will discuss specific agents commonly used in psychoneuroimmunology, starting with immunostimulatory agents, since they have been the most used in psychoneuroimmunology research.
Lipopolysaccharides

Theoretically, any molecule that is foreign to the organism has the potential to elicit an immune response. Nevertheless, polysaccharides are among the most potent molecules known to be capable of activating a strong immune response that is generally dose-dependent and usually results in death at high concentrations. Unequivocally, the most widely used antigen in psychoneuroimmunology has been lipopolysaccharides (LPS) from Gram-negative bacteria. In particular, a few serotypes obtained from *Escherichia coli* (E. coli), serotype O55:B5, serotype O127:B8 and serotype O111:B4 from Sigma (Sigma-Aldrich, St. Louis, MO), have been employed in a majority of studies. They have been shown to elicit a strong inflammatory response in almost all strains of rats and mice tested so far. An equivalent of LPS from *E. coli* obtained from *Salmonella abortus-equii* (S. abortus-equii) has been employed in a landmark clinical study administered intravenously at low concentration in healthy individuals under experimentally controlled conditions (Reichenberg *et al.*, 2001).

Polysaccharides are molecular components of cell walls of bacterial organisms. In particular, *E. coli* is an enterobacteriaceae living in the digestive tract of most mammals. The purified fraction of LPS (the O antigen from the above-mentioned serotypes) from *E. coli* is capable of inducing an immune response when present in systemic compartments of the organism. LPS binds to the soluble lipopolysaccharide-binding protein (LBP) forming the LPS–LBP complex, which in turn binds to CD14 (cluster of differentiation 14) and TLR-4. TLR4 activation leads to a well-described intracellular signaling cascade resulting in activation of the transcription factor NF-κB and production of pro-inflammatory cytokines. As PRRs, the CD14 and TLR4 receptors play critical roles in the innate immune response. Thus, LPS induces a dose-dependent activation of the innate immune response involving macrophages and the production of cytokines such as IL-1β, TNF-α and IL-6 that may ultimately result in death by septic shock if the concentrations are too high. The lethal doses for rats and mice are about 20 to 50 mg/kg depending on the strain and species. Subseptic doses of LPS ranging from 0.1 to 2.5 mg/kg administered peripherally by intraperitoneal (i.p.) injections are capable of inducing the production of cytokines in the brain primarily by microglia and astrocytes, and perhaps even neurons, depending on the dose. Most of the components necessary for the recognition of LPS, including CD14 and TLR4, are expressed in the brain, and mechanisms transducing signals from the periphery to the brain regarding the presence of LPS have been extensively studied. The production of cytokines by brain cells has been associated with the behavioral signs of sickness and other behavioral changes including depressive-like behaviors and cognitive impairments. The mechanisms by which LPS induces neurobehavioral changes are an active area of psychoneuroimmunology research.

The models that have been employed using LPS in the study of behavioral and psychological outcomes relate to the dose and route of administration, number of LPS challenges (single, repeated or chronic) and developmental stage of the challenge. The most common model has been administration of subseptic doses of LPS by i.p. injection. This model of subseptic i.p. LPS intends to mimic an inflammatory process in the periphery involving an increase in circulating cytokines such as IL-1β, TNF-α and IL-6, and it can be interpreted as a model of general systemic inflammation in general. Another model is the direct injection into the brain of low doses of LPS by various methods including a single injection or constant delivery by minipumps. This model has been used to study different mechanisms of interaction between locally produced cytokines and neurons. A less common model employs administration of LPS
by intranasal delivery; this model results in an inflammatory process in the respiratory tract that mimics certain types of chronic or seasonal respiratory infections.

Despite the usefulness and validity of these LPS models, certain limitations have been observed. For example, administration of LPS i.p. and in the brain has been shown to cause tolerance to further LPS challenges, limiting its usefulness as a model of chronic or sustained inflammation. The model has also been questioned due to the lack of evidence that any chronic inflammatory disease or psychological disorder is related to the presence of bacterial LPS. Another problem when using these approaches is the observed variability in the inflammatory response to LPS within individuals of the same species. While the exact reason for this variability has not been fully elucidated, some possibilities have been related to factors such as time of day of the administration, hormonal status, and housing conditions, including hierarchy in the home cage. Despite these limitations it must be recognized that the LPS-challenge model is one of the leading models in psychoneuroimmunology research.

**Nucleic acids**

Nucleic acids of viral and bacterial origin are also potent activators of the innate immune response. The use of synthetic strands of RNA and DNA to elicit an immune response has proved to be a valid alternative to the use of purified viral and bacterial RNA and DNA. In particular, poly I:C is employed to mimic viral RNA, and phosphorylated and repeated CG sequences oligodeoxynucleotides (CpG ODNs) are used to mimic bacterial DNA in models similar to those described for LPS. Synthetic CpG ODNs activate the immune response by a mechanism engaging Toll-like receptor 9 (TLR9), an intracellular PRR. Thus, the mechanism of immune activation mediated by CpG ODNs is different from that mediated by LPS, yet it also results in activation of the innate immune response and the production of pro-inflammatory cytokines. An important difference is that CpG ODNs are capable of also activating T<sub>H1</sub> responses and the production of interferons in addition to pro-inflammatory cytokines. Briefly, immunostimulatory CpG ODNs initiate a cascade of cellular activation generally starting from B cells and plasmacytoid dendritic cells (pDC) followed by natural killer cells, T-cells and macrophages resulting in the production of pro-inflammatory cytokines and chemokines including IL-1β, IL-6, IL-18, and TNF-α and the T<sub>H1</sub> cytokines IFN-γ and IL-12 (Klinman et al., 2008). They have also been shown to induce cytokine expression in the brain in a manner similar to that of LPS when administered peripherally via i.p. injections. A number of studies have determined the inflammatory and immunostimulatory properties of CpG ODNs in the brain and nervous system, mostly in relation to anti-cancer therapies and infections. However, there has been limited research on the neuropsychological and behavioral effects of CpG challenges. In contrast, poly I:C has been widely employed and is currently one of the leading models for studying immune activation during prenatal and perinatal periods and its effects on the brain and behavior. It also has been employed in models using adult animals that receive repeated administrations in models of chronic fatigue. Poly I:C RNA activates the immune response through a TLR3-dependent-mechanism resulting in the production of pro-inflammatory and T<sub>H1</sub> cytokines. An important role for TLR3 receptors has also been proposed for inducing adaptive immune processes and protective immunity to viruses. Thus, poly I:C challenges are regarded as adequate models of immune activation in response to viruses. Since certain psychiatric diseases, including schizophrenia, are believed to encompass a neurodevelopmental insult possibly involving viruses, poly I:C, rather than LPS, is used in models of maternal immune activation during gestation. In this model pregnant rats or mice are
administered i.p. or intravenously with doses around 5 mg/kg over several consecutive days. The offspring is then evaluated on behavior at different developmental stages from early puberty to adulthood. Several neurophysiological and behavioral abnormalities have been documented in these animals that resemble some of the core features of major psychiatric diseases (Meyer and Feldon, 2012). A consideration of this model is that pregnant mothers receiving poly I:C often have reduced litters and/or early mortality depending on the magnitude of the challenge. In addition, the model does not evaluate other important components of a viral infection such as antigen processing of viral proteins and adaptive immune responses. However, the model is extremely useful in showing how activation of the host immune response during critical developmental periods of the CNS results in long-lasting and permanent alterations of brain function and behavior.

**Superantigens**

Superantigens (SAgs) are a special class of protein toxins that originated from the biological activity of certain viral and bacterial organisms. They are capable of activating T-cells by direct stimulation of the T-cell receptor, leading to a rapid activation and expansion of T-cells. This process increases the production of T-cell cytokines such as IL-2, IL-12 and IFN-γ, and also results in neurobehavioral changes (Urbach-Ross and Kusnecov, 2009). Although SAgs stimulate T-cells to proliferate and produce cytokines, the process does not include the classical mechanisms of antigen processing and presentation of the adaptive immune response. Thus, the name “superantigens” is related to the property of these molecules of “bypassing” this mechanism to directly activate T-cells in a non-specific manner. These molecules bind to or “are recognized” in specific invariant regions of the variable portion of the T-cell receptor (Vβ region). Therefore, they have the ability to stimulate several clones carrying the same invariant gene, resulting in oligoclonal activation and expansion that can constitute up to 10 to 20 % of the T-cell pool of the organism. The most employed SAgs in psychoneuroimmunology research are bacterial enterotoxins secreted from the Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*). They have been characterized by their capacity to activate a number of T-cells in mice, rats, and humans. There are a number of identified superantigenic enterotoxins (SE) from *S. aureus* that have been termed A, B, C, etc., resulting in a serologic classification as SEA, SEB, SEC and so on. In particular, two types, SEA and SEB, have been used in C57Bl/6 and BALB/c mice respectively in psychoneuroimmunology research. SEA and SEB administered by i.p. injections have been shown to result in a complex neurobehavioral mechanism involving activation of specific brain regions and elevations in the cytokines IL-1β, IL-2, IL-6, IL-10, IFN-γ and TNF-α. The behavioral repertoire affected by SEA and SEB has been related to increased anxiety and neophobia (Rossi-George et al., 2005). Remarkably, these effects have been shown to occur without any overt signs of sickness which occur following LPS and poly I:C challenges. Several brain regions have been shown to express the immediate early gene c-fos during SAgs challenge, indicative of neuronal activation. Some of these regions such as the paraventricular hypothalamic (PVH) and arcuate (Arc) nuclei and the central nucleus of the amygdala (CeA) points to neuroendocrine and limbic activation. It also results in elevation of endogenous glucocorticoids and activation of the hypothalamo–pituitary–adrenal (HPA) axis. Another differential feature of this model of T-cell activation with respect to the LPS model is that HPA-axis activation has been shown to be dependent on the cytokine TNF-α alone, as compared to the synergistic actions of IL1-β, IL-6 and TNF-α in the LPS
model. The specific activation of T-cells and their cytokines, the absence of malaise, and the engagement of specific brain regions provide an excellent model of T-cell-dependent immune activation that allows for the differentiation of specific mechanisms mediated by T-cells from other non-specific models such as the LPS challenge. This is particularly relevant for human studies because most chronic inflammatory diseases are maintained by the activity of T-cells. Since most chronic inflammatory conditions have been associated with increased incidence of depressive and anxiety disorders, this model is a valid approach in psychoneuroimmunology research.

Proteins

Most protein complexes do not have antigenic properties for their recognition by innate immune cells or PRRs. This implies that a protein complex or a polypeptide that is strange to the organism will require antigen processing and presentation by dendritic cells to lymphocytes, a hallmark mechanism of adaptive or acquired immunity. This process will result in activation of a specific subset of lymphocytes that will initiate an immune process directed at neutralizing and/or clearing the pathogen containing the specific peptide sequence. It usually involves production of antibodies by B-cells and the establishment of immunological memory. The models employing proteins to induce an immune response require a protocol of induction that involves several steps, including a sensitization process by repeated exposure followed by antigen challenge after a period of time. Several proteins have been employed in such models including myelin basic protein (MBP) and albumin from chicken egg or ovalbumin (OVA). MBP is used to model autoimmune multiple sclerosis (MS) in a model initially called experimental allergic encephalomyelitis and later re-named experimental autoimmune encephalomyelitis (EAE), and represents one of the most widely used models to study the initiation and progression of MS. The few studies that have evaluated the emotional, cognitive, and behavioral implications of an active autoimmune and neurodegenerative process in the brain in the EAE model have confirmed that emotional and cognitive disturbances are associated with the inflammatory process of EAE. However, the number of studies addressing emotion and cognition in the EAE model are far less common than those focusing on mechanisms of the disease.

The second most common protein used for inducing and adaptive immune response is OVA, which has been extensively used as a model of protein antigen \textit{in vivo}. The immunization protocol involves sensitization by i.p. injections of OVA solutions coupled to an adjuvant and later exposure to the OVA peptide via respiratory, cutaneous or oral administrations. In general, this model has been employed to induce a $T_{H}2$-mediated inflammatory allergic response to OVA that, depending on the route of exposure, may mimic food, respiratory, or skin allergies. Challenge with OVA in sensitized mice and rats results in a complex $T_{H}2$-mediated inflammatory reaction that has become one of the standard models of experimentally induced allergies. It includes the clonal expansion of OVA-specific $T_{H}2$ lymphocytes and the production of IgE antibodies against OVA by B-cells. This leads to hypersensitivity to OVA that is mostly mediated by mast cell degranulation as the result of binding to and activation of the Fc receptor by the OVA/IgE complex. The inflammatory process triggered by the mediators released by mast cells, such as proteases, leukotrienes, histamine, etc., is amplified by the recruitment of eosinophils and basophils that produce and release more cytokines and inflammatory molecules. A feature of this model is that the magnitude of the inflammatory
response depends mostly on the number of challenges rather than the dose of antigen used. Thus, the inflammatory response increases with the number of exposures and not by the single exposure to a higher concentration of the antigen. It has been shown in mice and rats that allergies to OVA are paralleled by increased responses of anxiety in several behavioral tests, and that these responses are related to both early events of the allergic reaction such as IgE-dependent mast cell degranulation and also to later processes such as lymphocyte recruitment. The pattern of cytokine production corresponds to the T\(_{H2}\) type involving IL-4, IL-5 and IL-13 production mainly by T-cells, eosinophils, and basophils and eventually the release of TNF-\(\alpha\) from mast cells. Similarly to the SAgs model, allergies to OVA have been shown to result in c-fos expression in the PVH and CeA, and also in the nucleus of the solitary tract (NTS), suggesting a role for these regions in responding to processes mediated by T-cells (Costa-Pinto et al., 2005). In addition, exposure to OVA in sensitized animals does not result in overt signs of sickness, which is also a feature in the SAgs model. However, no clear evidence of elevations in endogenous glucocorticoids has been reported, pointing to important differences with the previously discussed models (Tonelli et al., 2009). The significance of this model also relies in the opportunity for clinical research since allergies are very common in the population and several human studies have established the link between allergies and anxiety disorders and behavioral responses of anxiety. For instance, state and trait anxiety in allergic individuals have been documented in clinical studies, and increased anxiety and emotional reactivity has been reported after antigen exposure in humans (Buske-Kirschbaum et al., 2008; Rosenkranz et al., 2005). Thus, the OVA model closely represents the emotional alterations associated with allergies – one of the most common chronic inflammatory conditions in the developed world.

Cytokines

The notion that specific cytokines are responsible for eliciting specific neurobehavioral responses through their direct interaction with different neural circuitries has been a major focus of research in psychoneuroimmunology. For instance, cytokines are produced within neural circuitries, and receptors for cytokines are expressed on neurons and other brain parenchymal cells (eg., glia), which can mediate consequent electrophysiological and intracellular responses. Thus, it is easy to understand that a major approach in psychoneuroimmunology has been the direct infusion of cytokines and their antagonists in different brain regions to study behavioral responses to pharmacological doses of cytokines. In addition, peripheral administration of cytokines via intravenous injections has also been extensively employed. These approaches have provided important evidence about the role of several cytokines in mediating behavioral responses associated with immune activation. For example, blocking IL-1\(\beta\) and TNF-\(\alpha\) actions in the brain prevents sickness behavior after LPS administration. These studies also showed that elevations of cytokines such as IL-1\(\beta\) in the periphery also result in the behavioral changes of sickness, and that blockade of IL-1\(\beta\) in the brain abolished the effect. Thus, these studies provided evidence that immune activation initiated in the periphery that results in the elevation of circulating cytokines is responsible for behavioral changes that are mediated by the actions of cytokines in the brain (Anisman, Gibb, and Hayley, 2008; Dantzer et al., 2008,). An important consideration of studies using direct infusion of cytokines to evaluate behavior is that there are no “true” pharmacological antagonists for cytokines in the classical sense of displacing the ligand from the receptor by competitive binding. Thus, the most employed
method for blocking or interfering with cytokine action is by using endogenously produced antagonists such as soluble IL-1 receptor antagonist (IL-1Ra) to prevent the actions of IL-1β. These endogenous cytokine antagonists are often found commercially available from different sources specializing in the production of recombinant proteins. Another method for blocking cytokine action is by using antibodies capable of neutralizing their biological activity, commonly called neutralizing antibodies. Finally, the use of genetically engineered recombinant proteins such as the TNF-α receptor inhibitor Etanercept may become the tool of choice for future pharmacological studies.

Live infectious agents

The live infectious agents, including viruses, bacteria, and parasites, used to study their effects on brain function and behavior can be distinguished by those capable of invading the CNS and establishing permanent infections in the brain and those that proliferate largely in peripheral organs with limited neuroinvasion. Models of chronic CNS invasion mostly involve the use of neurotropic viruses and the parasite *T. gondii*. These models have been employed in studies related to psychiatric diseases such as schizophrenia and autism which have been historically related to viral and parasitic CNS infections. Several studies have provided important insights on the neural and behavioral consequences of permanent or chronic CNS infections. For example, chronic infections in mice and rats with herpes simplex virus (HSV) and Borna disease virus (BDV) have been shown to produce specific alterations in the brain and behavior that relate to higher cognitive function such as learning and memory (Lipkin and Hornig, 2004). The model of chronic infection using *T. gondii* has revealed that the presence of the parasite in brain tissue is capable of influencing specific behaviors of fear and anxiety (Vyas et al., 2007) however the relationship of *T. gondii* infection and schizophrenia remains elusive. These models differ from other models of acute infection using the same and other viruses in the magnitude of the inflammatory response to an acute infection. For example, acute BDV and influenza infections may result in encephalitis with varied damage to the brain. Infections with influenza virus via intranasal instillations in pregnant dams have been employed in models of neurodevelopmental maternal viral insult (Patterson, 2009). The offspring of mice and rats infected with different strains of influenza during pregnancy display deficits in exploratory behavior, social interaction, and recognition of novel objects, and specific deficits in acoustic startle responses, similar to those observed in humans with schizophrenia. In these models the viral infection does not reach the fetus or the developing CNS and thus evaluates the effect of maternal immune activation on the offspring. Experimental infection with the live virus closely represents the course and progression of illness and therefore it may be a better model than poly I:C when studying interactions of maternal immunity and brain development. However, the poly I:C model is more commonly used owing to the more direct set up and implementation of the model. Finally, a model of live bacterial infection has been used by inoculating mice with i.p. injections of an attenuated form of *Mycobacterium bovis*, bacilli Calmette-Guerin (BCG) (O’Connor et al., 2009). Infection of mice with BCG results in a progression of illness characterized by an initial stage during which symptoms of sickness behavior manifest; this is followed by their resolution during the next stage although the mycobacteria are still present in organs such as the lungs, liver and spleen. During this time, cytokine expression and cellular immunity remain activated, offering a window of opportunity to conduct behavioral tests and thus representing a good model of chronic inflammation and/or immune activation.
Basic Principles in Immunology

Immune Processes and their Clinical Consequences on Mental Health

The models presented here have been and continue to be actively employed to evaluate the potential involvement of immunity in initiating, precipitating or perpetuating complex psychiatric diseases of unknown etiology. Although the causes leading to the pathophysiology of mental illness remain unresolved, there is a significant body of evidence implicating the immune system and/or inflammatory processes in some aspects of anxiety and depressive disorders, schizophrenia, and autism as well as in the cognitive and psychological decline of aging. For example, interest in the psychoneuroimmunology of depression was sparked by the observation that cancer patients receiving interferon-alpha (IFN-α) therapy would often develop depressive symptoms. Numerous studies since then have shown that depression in humans is associated with inflammation. Over many studies, researchers have determined a general immunological profile of depression generally characterized by increased acute phase proteins and impaired cellular immunity as well as increased inflammatory cytokines such as IL-1β, IL-6, and TNF-α (Blume, Douglas, and Evans, 2011), and a mild inflammatory response (Kronfol, Singh, and Zhang 1995). Dysregulation of the HPA axis is one of the most consistent physiological findings in major depression; specifically, corticotrophin-releasing hormone (CRH) hypersecretion accompanied by a lowered adrenocorticotropic hormone (ACTH) response to CRH stimulation. Pro-inflammatory cytokines such as IL-1β and IL-6 stimulate the production of CRH and thus may be driving this dysregulation (Maes et al., 1993). Depression in humans encompasses a broad range of symptoms; some of them, including fatigue/hypersomnia, insomnia, weight gain, weight loss, irritability, anhedonia, lack of energy, and decreased libido, are measurable in animals during activation of the innate immune response. The suite of behavioral changes that accompany the LPS model, including social withdrawal, altered sleep patterns, reduced food and water intake, as well as lower activity levels, that collectively are called sickness behavior can be used, with the appropriate considerations, as a proxy for depression. Thus, the behavioral depression induced by the LPS model provides a working model to study mechanisms of neuroimmune interaction leading to the worsening of the motivational state of mammalian organisms (Dantzer et al., 2008).

Another example of the validity of the models discussed previously is the association between T-cell function and anxiety disorders, a large category encompassing a number of distinct disorders, including generalized anxiety, specific phobias, social phobia, post-traumatic stress disorder (PTSD), obsessive–compulsive disorder, and panic disorder. As discussed above, anxiety disorders have been linked to allergic and autoimmune disorders by a significant number of epidemiological and clinical studies. Thus models based on T-cell activation, such as the OVA model, which induce responses of anxiety provide a good working model to study the relationship between allergic inflammation, T-cell function, and anxiety disorders. The SAgS model that involves specific T-cell responses and also affects anxiety provides an additional model to study this relationship. Furthermore, severe anxiety syndromes such as PTSD are accompanied by immunological alterations affecting lymphocyte function. A known long-lasting physiological consequence of psychological trauma is the one observed on T lymphocyte number and function. A number of studies report a higher lymphocyte count in PTSD patients. Findings include increased total lymphocytes (Boscarino and Chang, 1999; Vidovic et al., 2007), higher percentage of cytotoxic CD8+ T-cells (Lemieux, Coe, and Carnes, 2008) and increased overall
CD8+ cytotoxic T-cells (Skarpa et al., 2001). These findings suggest increased production of T-cells with an inflammatory profile (increased cytotoxic and memory-effector T-cells) which last for a considerable time after exposure to psychological trauma and are associated with the permanence of PTSD symptoms (Gill et al., 2009). In addition, natural killer cell activity varies with temporal proximity to trauma, being increased in chronic PTSD and reduced in recent trauma survivors (Vidovic et al., 2007). Circulating inflammatory cytokines, specifically IL-6 and TNF-α, are higher in people suffering from PTSD than in traumatized non-PTSD controls (Maes et al., 1999). Interestingly, this higher circulating IL-6 may be a predisposing factor to the development of PTSD (Sutherland, Alexander, and Hutchison, 2003). In sum, there is a significant parallel between altered lymphocyte function and anxiety disorders that warrants the interest of psychoneuroimmunology.

Further interest in the role of immunity in mental health stems from the evidence of a possible etiology for schizoaffective disorders linked to an infectious agent during development. Studies demonstrated a significant susceptibility in people born in the winter, when rhinoviruses and influenza are most active; population studies have shown that schizophrenia cases also increase after influenza epidemics. Later studies have shown that prenatal exposure to influenza, toxoplasmosis, and herpes viruses like herpes simplex, Epstein-Barr virus, and cytomegalovirus are associated with offspring schizophrenia. These diverse findings indicate that viral infection in general, rather than any one specific pathogen, may be the immune trigger for the long-lasting damage seen in schizophrenia. In this regard, the poly I:C model offers a valuable tool to study specific mechanisms of viral immune activation during pregnancy and their consequences in adulthood on the brain and behavior. It produces schizophrenia-like alterations in the brain, and the associated behavioral symptoms respond to proven anti-psychotic drugs (summarized in Meyer and Feldon, 2012). Additional major psychiatric diseases linked to a developmental immune insult are autism spectrum disorders. A large epidemiological study in Denmark found a significant association between maternal viral infection in the first trimester and autism (Atladottir et al., 2010). In this case, a role for the production of maternal antibodies against viral or bacterial infections has been proposed. Experiments on antibody transfer provide compelling evidence for this hypothesis. In this type of study, antibodies are collected from mothers who have children with autism, which are then injected into a pregnant animal. In both mice and rhesus monkeys, the resultant offspring have been shown to develop autism-like behaviors, such as hyperactivity, stereotypy, and impaired social interaction (Singer et al., 2009). These fetal-brain-specific IgG antibodies are associated with approximately 15% of human autism cases, and seem specific to the regressive type of autism, in which a child meets developmental milestones until the age of two years, and then begins to miss them.

Concluding Remarks

This chapter presented a brief introduction to some basic aspects of immunity and how it relates to brain function and behavior in the context of mental health research. It also presented some of the models used to study the effects of activation of the immune system on emotional and cognitive function. As these models are dynamic and continue to improve, it is expected that better models of psychoneuroimmune interaction will shed light on complex psychiatric diseases and perhaps provide targets of improved intervention to treat these diseases.
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