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CHAPTER

33

Molecular Mechanisms and Consequences of Immune and Nervous System Interactions

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OUTLINE

INTRODUCTION

Definition: What is neuroimmunology?

The immune system plays two essential roles necessary for the survival of complex organisms (Lo et al., 1999; Carson et al., 2006; Goverman, 2009; Schwartz & Kipnis, 2011; Dilger & Johnson, 2008):

- maintaining tissue homeostasis
- providing tissue defense against pathogens.

These immune functions are essential to maintain the functions of all organs in the body and are studied as part of the general field of immunology. Neuroimmunology is the specific study of the interactions between the nervous system and the immune system as well as the cross-regulatory impacts of these interactions on both immune and nervous system functions (Lo et al., 1999; Carson et al., 2006; Goverman, 2009; Schwartz & Kipnis, 2011; Dilger & Johnson, 2008).

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Scope: Are neuroimmune interactions relevant only in the context of immune-mediated neurodegenerative disorders?

For most of the 20th century, neuroimmune interactions were largely studied and characterized for their detrimental effects on nervous system function and for their contributions toward the onset and progression of neurodegenerative disease, autoimmunity and exacerbation of injury-induced loss of neuronal function (such as in spinal cord injury) (Carson et al., 2006; Goverman, 2009; Schwartz & Kipnis, 2011; Dilger & Johnson, 2008). In part, the predominant research focus on immune-mediated neurotoxicity is a consequence of the following common (but incorrect) presumptions:

- The nervous system played little to no role in regulating immune responses and was thus passive and always on the receiving end of neuroimmune interactions.
- Immune functions were primarily aimed toward pathogen defense and were thus always cytotoxic.
- Neuronal function was incompatible with exposure to activated immune cells and their pro-inflammatory products.
- Therefore, to maintain CNS function of post-mitotic neurons, it was essential that the CNS be isolated from the immune system by the blood–brain barrier (BBB) and thus protected from cytotoxic substances produced by activated immune cells.

In this chapter, we will discuss the limitations inherent in the statements above. Specifically, we will detail multiple mechanisms by which the nervous system regulates and directs immune function toward what is needed and tolerated by the nervous system. Identification of multiple regulatory points also suggests multiple sites that can be disrupted by pathogens, toxins or genetic abnormalities to trigger, facilitate and/or exacerbate the onset and progression of classic neurodegenerative disorders. Surprisingly, targeted disruptions in T-cell and macrophage functions have revealed previously unknown essential roles of the immune system in maintaining cognitive function even in the absence of overt infection and injury (Schwartz & Kipnis, 2011; Dilger & Johnson, 2008; Derecki et al., 2010; Chen et al., 2010). For example, CD4+T-cell deficiency in murine models leads to decreased performance in tests of murine learning and memory (Derecki et al., 2010). In addition, targeted disruption of Hoxb8 in macrophage lineages is sufficient to trigger onset of obsessive-compulsive–like behaviors in otherwise healthy mice free of pathogenic infection or injury (Chen et al., 2010). Supplementation of Hoxb8-deficient mice with wild-type bone marrow was sufficient to restore normal behavior (Chen et al., 2010).

Relevance: A real-world example

The relevance of understanding neuroimmune interactions is not limited to select populations suffering specific neurodegenerative or rare genetic disorders. T-cell–dependent control of the human pathogen *Toxoplasma gondi* (*T. gondii*) illustrates that the sustained chronic influx of activated, IFNγ-producing lymphocytes into the CNS can be well tolerated without causing overt neurodegeneration (Ferguson, 2009; Wilson et al., 2010). *T. gondii* is an obligate intracellular protozoan able to infect all mammals. Humans generally become infected due to ingestion of undercooked infected meat or exposure to *T. gondii* sporozoites in soil. Once an individual is infected by *T. gondii*, the parasite invades all tissues in the body. An individual's immune system is able to rapidly clear the fast-replicating tachyzoites from most tissues, but cannot clear all forms of the parasite from the body. Instead, *T. gondii* evades elimination by the immune system from sites of tissue infection by entering the latent bradyzoite tissue cyst stage of its life cycle. Lifelong influx of interferon-gamma (IFNγ)–producing T-cells into tissues is required to prevent emergence of the cytotoxic tachyzoites from latent tissue cysts.

Approximately 30% of the world's population (~10–20% of the population in the United States, ~80% of the population in France and Brazil) is estimated to have chronic CNS infections of *T. gondii* (Ferguson, 2009; Wilson et al., 2010). In adult humans with competent immune systems, infection by this common pathogen is usually benign. By contrast, in humans with severe immunodeficiency (such as that caused by HIV or chemotherapy), the failure of sufficient numbers of IFNγ-producing T-cells to infiltrate the CNS is accompanied by emergence of cytotoxic tachyzoites from tissue cysts and widespread necrotizing damage to the CNS. Left untreated, this condition can be fatal (Ferguson, 2009; Wilson et al., 2010). Despite the presence of IFNγ-producing T-cells and activated macrophages in their brains, immune-competent individuals infected with *T. gondii* do not display overt clinical or histologic signs of neuropathology such as those associated with multiple sclerosis, Alzheimer's disease or Parkinson's disease despite chronic CNS inflammation (Carson et al., 2006; Dilger & Johnson, 2008; Ferguson, 2009; Wilson et al., 2010). It is possible that subtle changes in brain function result from this type of chronic inflammation. However, the behavior of the population as a whole in regions with high prevalence of *T. gondii* infections clearly indicates that high levels of cognitive and motor brain function can be maintained in the presence of at least one common form of lifelong brain inflammation.

These examples indicate that properly regulated neuroimmune interactions can be highly adaptive and necessary to support nervous system function in a non-sterile world (Lo et al., 1999; Carson et al., 2006; Goverman, 2009; Schwartz & Kipnis, 2011; Dilger & Johnson, 2008; Siffrin et al., 2007). However, this does not minimize the potential neurologic damage and clinical dysfunction caused by aberrant or dysregulated neuroimmune interactions. In particular, CNS function is dependent on large populations of post-mitotic neurons and experience-driven formation of synaptic pathways. Therefore, it is vulnerable to functional disruption caused by pathogen-mediated damage in the absence of sufficient immune responses, as well as by immune-mediated damage in the presence of overly robust immune responses. However, as in the case of suspected immune-mediated triggers of autism, it is not always clear if the neuronal dysfunction is caused by too few, too many or just the wrong types of neuroimmune interactions (Dilger & Johnson, 2008; Brynskikh et al., 2008).

In following sections, we define the differential roles of cells in the nervous and peripheral immune systems

in determining the types of acute and chronic inflammation triggered by pathogens, injury and tissue homeostasis. We will also focus on defining neuroimmune mechanisms that regulate and trigger "just enough" of the right type of immunity required to maintain CNS function in a non-sterile world.

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Innate versus adaptive immunity: two interacting types of immune recognition

The immune system is divided into two arms: a rapid "innate" immune response system and a slow "adaptive" immune response system (Lo et al., 1999). The receptors for the types of "alarm" signals detected by the innate immune system are preformed and stably encoded in the genome. By contrast, the receptors for the types of signals that trigger the adaptive immune system are in part stochastically generated and in part shaped by the types and frequency of pathogens encountered.

Innate immunity is triggered by evolutionarily conserved alarm signals

The innate immune system consists primarily of neutrophils, macrophages, eosinophils, mast cells and other granulocytes as well as specialized natural killer cells (Lo et al., 1999; Carson et al., 2006; Goverman, 2009; Schwartz & Kipnis, 2011; Siffrin et al., 2007). In the adult mammal, these cells are short-lived and replaced by bone marrow–derived stem cells. Innate immune cells are considered "first responder" cells that rapidly recognize and respond to the following conserved molecular patterns: danger associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs).

DAMPs are molecules that are released or expressed only when cells are damaged or infected. For example, macrophages recognize dying cells by using purinergic receptors to detect the release of purines and scavenger receptors to detect the appearance of phosphatidylserine (PS) on the extracellular surface of the plasma membrane. In healthy cells, PS is actively sequestered to the internal cytoplasmic face of the plasma membrane, while apoptotic and necrotic cells lose the ability to sequester PS. Not surprisingly, DAMP-mediated activation of innate immune cells promotes increased phagocytic activity.

PAMPs are evolutionarily conserved molecules expressed by pathogens but not by host cells. For example, neutrophils and macrophages can detect the presence of invading gram-negative bacteria via toll-like receptor (TLR)–4 binding of lipopolysaccharide (LPS). LPS is a component of gram-negative bacterial cell walls that is not expressed by mammalian cells. PAMP-mediated activation generally leads to amplified pro-inflammatory responses by innate immune cells. These responses include production of cytotoxic reactive oxygen species, nitric oxide and pro-inflammatory cytokines such as TNF, IL-1 and IL-12.

Adaptive immunity can recognize evolutionarily novel molecules

Molecules expressed by pathogens often undergo rapid mutation as pathogens attempt to evade detection and thus elimination by the infected host's immune system (Lo et al., 1999; Carson et al., 2006; Goverman, 2009; Schwartz & Kipnis, 2011; Siffrin et al., 2007). If the immune systems could only detect evolutionarily conserved molecules, pathogens would readily be able to evolve to evade immune-mediated elimination. Thus, the vertebrate immune system has a second arm referred to as the adaptive immune system, which is able to detect and respond to novel as well as evolutionarily conserved pathogenic molecules (Lo et al., 1999; Carson et al., 2006; Goverman, 2009; Schwartz & Kipnis, 2011; Siffrin et al., 2007). Molecules able to stimulate adaptive immune responses are called antigens.

Lymphocytes (T-cells and B-cells) are long-lived cells that together with antigen-presenting cells comprise the adaptive immune system (Lo et al., 1999; Carson et al., 2006; Goverman, 2009; Schwartz & Kipnis, 2011; Siffrin et al., 2007; Korn et al., 2007). Immature T-cells and B-cells develop in the thymus and bone marrow, respectively, before being released into circulation. During this maturation process, the specific genomic cassettes that encode the ligand-binding pockets of their T-cell and B-cell antigen receptors (TCR and BCR respectively) are stochastically rearranged in a process termed somatic recombination. The primary consequence of somatic recombination is that all TCRs or BCRs expressed by an individual T- or B-cell will have the identical antigenic binding pocket. Because somatic recombination is an irreversible rearrangement of DNA, only clonal progeny generated from the same lymphocyte progenitor will share the same rearranged antigenic receptor.

Antigen-driven expansion of clonal populations of lymphocytes is the reason that the lymphocyte arm of the immune system is termed the adaptive immune system (Lo et al., 1999; Carson et al., 2006; Goverman, 2009; Schwartz & Kipnis, 2011; Siffrin et al., 2007; Korn et al., 2007). Experience shapes the antigenic repertoire recognized by the adaptive immune system to fit the pathogens most likely to be encountered in the host's environment. Only the few lymphocytes that express receptors able to recognize an infecting pathogen will proliferate and become activated. The immune system also "adapts" to any mutations that the pathogen may introduce to evade the host's immune system. As new antigenic targets are revealed by a pathogen, they trigger expansion and activation of additional lymphocyte populations. Because lymphocytes are long-lived, each pathogen encounter causes long-term increases in the frequency of memory lymphocytes able to attack the pathogen if re-encountered. Upon antigen re-encounter, memory lymphocytes have a lower activation threshold and a faster rate of activation. This phenomenon is termed immunologic memory and is the basis of vaccine-boosted immune responses.

Antigen presentation by major histocompatibility-complex–expressing cells is required to activate T-cells

There are two additional levels of regulation involved in the activation of the adaptive immune system (Lo et al., 1999).

First, while the B-cell receptor can recognize free antigens of multiple chemical compositions and trigger B-cell activation, by itself BCR-mediated activation is insufficient to cause robust antibody production. B-cell production of antibodies requires "help" from antigen-activated cytokine-producing CD4+T-cells in addition to BCR activation.

Second, T-cells cannot detect their target antigens without help provided by an antigen-presenting cell (Lo et al., 1999). For example, a T-cell expressing a TCR specific for *T. gondii* peptides cannot detect the presence of *T. gondii* peptides even if immersed in a solution of *T. gondii* peptides (Figure 33-1). Instead, a phagocytic cell such as a macrophage or dendritic cell must capture free-, debris-, cell- or pathogen-associated antigens and then load the captured antigen into the pocket of the major histocompatibility complex (MHC) molecules expressed on its surface. This MHC-expressing phagocytic cell is called an antigen-presenting cell (APC).

Chemoattractant cytokines called chemokines can recruit T-cells to sites of injury and infection (Siffrin et al., 2007; Brynskikh et al., 2008; Kim et al., 2010; Müller et al., 2007; Noor et al., 2010; Reboldi et al., 2009; Korn et al., 2007). However, T-cells will neither be functionally active nor remain at the sites of injury or infection unless activated onsite by antigen presentation (Lo et al., 1999; Carson et al., 2006; Siffrin et al., 2007). CD4+T-cells recognize the antigen only when presented in the pocket of MHC class II of an APC, while CD8 T-cells recognize antigen only in the pocket of MHC class I of an APC. Antigens presented by MHC class II are captured during phagocytosis, while antigens presented by MHC class I are synthesized by the protein synthetic machinery within the antigen-presenting cell.

Most cells do not constitutively express high levels of MHC class I, unless they are infected by intracellular pathogens such as *T. gondii*, are stimulated by pro-inflammatory cytokines or are pre-apoptotic (Lo et al., 1999). When cells are infected by an intracellular pathogen, the cell's protein synthetic machinery produces both self- and pathogen peptides. As a result, self-antigens and pathogen antigens will be loaded into the pocket of the MHC class I molecule as it travels to the plasma membrane. Once activated by antigen presentation, the functional response of CD8 T-cells is to kill the infected and/or severely damaged MHC class I–expressing cell (Lo et al., 1999; Carson et al., 2006; Siffrin et al., 2007). Consequently, activated CD8+T-cells are also referred to as cytolytic lymphocytes (CTLs). Nearly all cells including CNS neurons can be induced to produce MHC class I. However, it should be noted that neurons are not as readily induced to express MHC class I as other cells in the CNS, such as oligodendrocytes and astrocytes. It is speculated that the much higher resistance of neurons to expression of MHC class I is an adaptive measure to prevent CD8+ T-cell triggered lysis of infected but still functional CNS neurons (Carson et al., 2006; Dilger & Johnson, 2008; Siffrin et al., 2007).

By contrast, MHC class II expression is primarily restricted to immune cells with well-defined antigen-presenting functions, specifically B-cells, macrophages and dendritic cells (Lo et al., 1999). Of these cells, the dendritic cell is the most effective at initiating CD4+T-cell activation and is often termed the "professional" APC of the body. Dendritic cells play the primary role in the initiation of CD4+T-cell responses, especially of "naïve" (antigen-inexperienced and never before activated) CD4+T-cells. While MHC class II$^+$

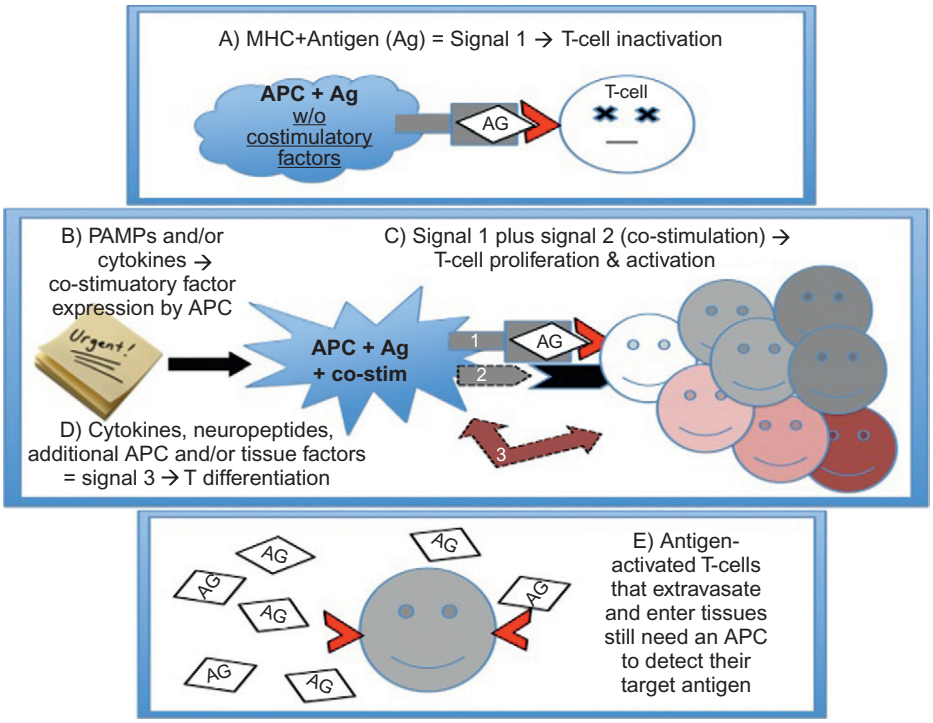

FIGURE 33-1 **The outcome of antigen presentation is dependent on T-cells receiving signals 1, 2 and 3.**

macrophages and B-cells can also activate naïve T-cells, they are much less effective than dendritic cells. Macrophages and B-cells do play critical roles in reactivating and directing T-cell activation within tissues and sites of antibody production.

Within the central nervous system, infiltrating and resident myeloid cells are the primary cells able to express MHC class II (Carson et al., 2006; Siffrin et al., 2007). Several other CNS cells have been reported to express some forms of MHC. For example, CNS neurons express non-classical MHC molecules that are demonstrated to play roles in the maturation of the visual system. However, these non-classical MHC molecules are not recognized to have antigen presentation functions. Astrocytes in glial cultures do express functional MHC class II. Because astrocytic expression of MHC class II within the intact CNS is not detected in healthy or disease conditions, this *in vitro* expression appears to be an aberrant consequence of culture. Therefore, neurons, astrocytes and oligodendrocytes do not play roles in antigen-mediated activation or retention of CD4+T-cells within the intact CNS.

Antigen-activated T-cells regulate the activation of innate immune cells

Following antigen presentation by MHC-expressing APCs, CD4+T-cells are stimulated to differentiate into one of a broad array of phenotypes (Lo et al., 1999; Goverman, 2009; Siffrin et al., 2007; Korn et al., 2007). Based on their production of factors that determine and support specific activation states in innate immune cells and B-cells, antigen-activated T-cells CD4+T-cells are referred to as T helper (Th) cells. CD4+T-cells phenotypes range from pro-inflammatory (Th1, Th17), humoral (Th2, Th9, ThFH), and anti-inflammatory (Th3, T regulatory [Treg]) to even anergized (permanently inactivated) phenotypes (Figure 33-1). IFNγ-producing Th1 CD4+T-cells promote myeloid cells to develop classical activation state (also termed M1) characterized by enhanced microbicidal and tumoricidal abilities, high expression levels of INOS, CD40, MHC class I, MHC class II and robust secretion of pro-inflammatory cytokines (IL-1β, IL-12, IL-23 and TNF-α) (Colton & Wilcock, 2010; Gordon & Martinez, 2010; Graeber & Streit, 2010; Yang et al., 2010). Conversely, Il-4– and IL-13–producing Th2 CD4+T-cells are not only essential for B-cell activation, Th2 cells also promote alternative activation states (also termed M2a and 2b) in myeloid cells. Alternative activation states are characterized by expression of macrophage mannose receptor (also referred to as CD206), YM1, stabilin-1 and arginase I. Alternatively activated cells are functionally associated with enhanced phagocytosis, tissue repair and parasite elimination. Finally, Il-10-, TGFβ-producing Th3 cells and Treg cells promote regulatory activation states (also termed M2c) in myeloid cells. These states are characterized by production of molecules associated with tissue repair and immunosuppression (Colton & Wilcock, 2010; Gordon & Martinez, 2010; Graeber & Streit, 2010; Yang et al., 2010).

The activation state of the antigen-presenting cell regulates T cell activation and phenotype

The type of T-cell phenotype that is induced by antigen presentation is largely dependent on the co-stimulatory molecules and cytokines expressed by the antigen-presenting cell (Lo et al., 1999; Carson et al., 2006; Goverman, 2009; Schwartz & Kipnis, 2011; Siffrin et al., 2007). Thus, classically activated macrophages and dendritic cells preferentially promote Th1 and Th17 differentiation of CD4+T-cells, while alternatively activated myeloid cells preferentially promote differentiation of T-cells with humoral and anti-inflammatory phenotypes. To summarize, the interactive cross-regulation between the innate and adaptive immune systems can appear circular, resulting in either beneficial or detrimental outcomes for the host. This cross-regulatory aspect of innate and adaptive immunity can lead to increased refinement of the immune response such that the pathogens (and thus all antigenic and alarm stimuli) are eliminated and immune resolution and tissue repair programs are effectively launched. Alternatively, chronic inflammation results if the pathogen or pathogen associated antigenic stimuli (such as unclearable pathogenic toxins or cross-reactive self-antigens) cannot be eliminated. Chronic inflammation can be highly detrimental to tissue function due to immune-mediated cytotoxicity and dysregulated fibrotic repair.

Choosing between immune tolerance and inflammation

Because TCRs and BCRs are generated from stochastic rearrangement of genomic cassettes within the TCR and BCR genes, there is a high potential for generating antigen receptors that recognize normal "self" molecules. Triggering apoptosis in autoreactive lymphocytes after they have completed somatic recombination, but before they are released from the

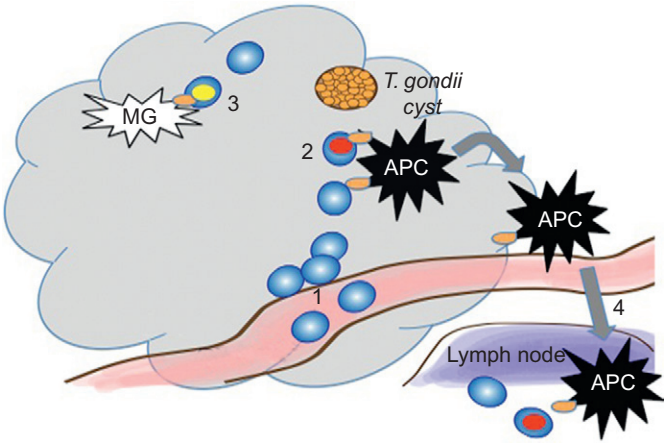

FIGURE 33-2 **Antigen presentation within the CNS. (1)** T-cells and peripheral antigen-presenting cells (APCs) enter the CNS in a two-step procedure. They first extravasate across the blood brain barrier into perivascular spaces. Then, chemokine production by astrocytes, neurons and microglia provides guidance cues for T-cell migration from the perivascular sites into and within the brain parenchyma. **(2)** MHC+, co-stimulatory molecule+blood-derived APCs present antigen to infiltrating T-cells, in this case promoting IFNΥ production by *T. gondii* antigen-specific T-cells. **(3)** Activated microglia express different sets of co-stimulatory molecules and prime antigen-specific T-cells to develop neuroprotective functions **(4)** Antigen-loaded blood derived APCs, but not microglia, can leave the CNS and return to peripheral lymphoid organs to activate additional antigen-specific T-cells.

thymus and bone marrow, prevents large numbers of high-affinity autoreactive lymphocytes from being in circulation (Lo et al., 1999; Carson et al., 2006; Goverman, 2009; Siffrin et al., 2007). The process of eliminating autoreactive T-cells is dependent on the expression and presentation of self molecules by MHC-expressing cells in the thymus. Elimination of autoreactive T-cells in the thymus process is not 100% efficient. Autoreactive T-cells with moderate and low affinity TCRs do escape deletion.

Antigen presentation in the absence of alarm signals promotes tolerance

Immature dendritic cells and/or tissue macrophages populate all tissues in the body, including the CNS (Lo et al., 1999; Carson et al., 2006; Goverman, 2009; Schwartz & Kipnis, 2011; Colton & Wilcock, 2010; Gordon & Martinez, 2010; Graeber & Streit, 2010; Yang et al., 2010). In the uninfected healthy individual, these immature dendritic cells and tissue macrophages routinely contribute to tissue homeostasis by the phagocytosis of cell debris. In doing so, these tissue APCs capture self-antigens and load them into their MHC class II molecules. Once activated by phagocytosis, these antigen-presenting cells migrate to the lymph nodes draining each tissue. In the lymph node, these short-lived APCs move among the large number of T-cells within the lymph node, testing if any have TCRs able to recognize their captured self-antigen. Soluble tissue antigens also reach lymph nodes by passive drainage along the lymphatic system associated with most tissues. MHC-expressing dendritic cells take up these antigens and present them to T-cells within the lymph node. If these antigen-presenting cells do not encounter other activating stimuli such as PAMPs or pro-inflammatory cytokines, they will express MHC but no other co-stimulatory factors. Consequently, they will only provide what is referred to as "signal 1" to a T-cell, with the result that the T-cell is permanently inactivated. To restate, mature naïve T-cells activated by their TCR and no other co-stimulatory signals will become permanently inactivated (referred to as anergized). This ongoing process of presenting self-antigens in the absence of alarm signals, resulting in the inactivation of autoreactive T-cells, is termed *peripheral tolerance*.

PAMP and DAMP signals shape APC function and T-cell differentiation

By contrast, APCs begin to express a wide array of co-stimulatory molecules when activated by PAMPs and pro-inflammatory stimuli during the process of capturing antigens (Lo et al., 1999; Carson et al., 2006; Goverman, 2009). When occurring during the process of MHC-mediated antigen-presentation, co-stimulatory factors provide signals 2 and 3 of T-cell activation. Signal 2 promotes T-cell proliferation and survival, while signal 3 determines T-cell differentiation and function (Figure 33-1). Antigen-induced T-cell functions can range from pro-inflammatory to anti-inflammatory depending on the types and concentrations of the molecules providing signals 2 and 3 (see "Antigen-activated T-cells regulate the activation of innate immune cells" in this chapter). Following antigen activation, T-cells leave the lymph nodes and infiltrate tissues throughout the body. However, as previously stated, these tissue-infiltrating, antigen-activated T-cells cannot detect their target antigens upon re-encounter unless there are antigen-loaded APCs within tissues.

THE NERVOUS SYSTEM REGULATES BOTH INNATE AND ADAPTIVE IMMUNITY

The whole of the preceding section can be summarized briefly as follows: Evolutionarily conserved alarm signals lead to rapid activation of the innate immune system within minutes to hours serving to defend the body for the ~96 hours required to activate the adaptive immune system. The specific combination of PAMP, DAMP and cytokine receptors triggered will determine the types of immune cells recruited to the site of infection or injury as well as the type and degree of their antigen-presenting cell functions. The activation state of these APCs will in turn determine the differentiation state of antigen-activated T-cells, which in turn recruit and activate the next wave of innate immune cells. This cycle keeps repeating until the offending stimuli can no longer be detected. One critical element missing from this summary is the role of the peripheral and central nervous systems in regulating the activation threshold of immune cells as well as their propensity to acquire pro versus anti-inflammatory phenotypes.

Functional consequences of lymphoid tissue innervation

Both primary (thymus and bone marrow) and secondary (lymph nodes and spleen) lymphoid tissues exhibit a high degree of sympathetic innervations (Nance et al., 2007; Gonzalez–Rey et al., 2010). Norepinephrine (NE) is the primary neurotransmitter released from sympathetic neurons. Most lymphocytes express a wide variety of neurotransmitter receptors. However, the level of expression differs as a function of activation state and cell type. For example, the β2 adrenergic receptor (receptor for NE) is expressed at much higher levels in Th1 than in Th2 CD4+T-cells. Thus, β2 adrenergic agonists preferentially induce cAMP elevations in Th1 CD4+T-cells over Th2 CD4+T-cells and play a larger role in regulating Th1 function. The extent to which the nervous system can direct and shape adaptive immune responses is illustrated by studies quantifying the effects of denervating the lymphoid organs by mechanical or chemical methods. For example, in animal models with normal innervation, Th1 CD4+T-cells promote B-cell production of IgG1 antibody isotypes, while Th2 CD4+T-cells promote B-cell production of IgG2a antibody isotypes. Denervation of the spleen leads to increased ratios of IgG2a to IgG1, indicative of an increase in Th2 CD4+T-cell responses and consistent with the *in vitro* observations of NE-mediated regulation of Th1 function. *In vitro* studies have also shown that direct stimulation of the β2 adrenergic receptor on B-cells increases the propensity to produce immunoglobulin (Ig) E, which is associated with allergic (type 1 hypersensitivity) and anti-parasitic immune responses (Nance et al., 2007; Gonzalez–Rey et al., 2010). Consistent with these observations, in animal models contusion models of spinal cord injury (SCI) have illustrated that altering neuronal activity to lymphoid organs alters

B-cell production of antibodies (Held et al., 2010; Lucin et al., 2007).

Lymphoid organs are innervated by sympathetic preganglionic neurons distributed throughout the thoracolumbar spinal cord. High-level SCI (at thoracic level 3) caused sustained increases in splenic NE along with impaired antibody synthesis and elevated splenocyte apoptosis that could be reversed by using β2 adrenergic receptor blockers (Lucin et al., 2007). Furthermore, impaired antibody production could be mimicked with low level SCI (thoracic level 9) with a beta2AR agonist. The effects of denervation are not limited to B-cells. *In vitro* and *in vivo* data reveal that NE inhibits the production of pro-inflammatory molecules such as TNF by lymphoid macrophages and dendritic cells. Innervation even affects the generation of mature lymphocytes. Seizure-associated changes in the sympathetic innervation of the thymus decrease the proliferative capacity of the lymphocytes being produced as well as changing the ratios of CD4+to CD8+T-cells that successfully mature (Nance et al., 2007; Gonzalez–Rey et al., 2010; Held et al., 2010; Lucin et al., 2007; Carson & Lo, 2007). This may in part explain why SCI injury at all levels decreases the ability to trigger anti-viral responses in both innate and adaptive immune cells.

Neuropeptides are potent modulators of antigen-presenting cell function

As stated above, neuropeptides can have stage-specific effects due to the selective expression of neuropeptide receptors (Gonzalez–Rey et al., 2010; Carson & Lo, 2007). Dendritic cells are considered to be the professional APCs of the immune system and critical for the initial activation and polarization of T-cell responses (see above). As a function of maturation, dendritic cells progress from being highly phagocytic cells with lower expression of MHC and co-stimulatory molecules to being relatively nonphagocytic cells with very high expression of MHC and costimulatory molecules (Lo et al., 1999). It is highly notable that coincident with their maturation into potent APCs, dendritic cells transition from expressing primarily serotonin 5-HT1B-, 5-HT1E- and 5-HT2B-receptors in their immature state to expressing primarily 5-HT4- and 5-HT7-receptors in their mature state (Gonzalez–Rey et al., 2010; Carson & Lo, 2007). Pharmacological studies demonstrate that the expressed receptors are functional and that they regulate stage-specific functions in dendritic cells. For example, 5-HT4–and 5-HT7–specific ligands suppress dendritic cell secretion of IL-12 and TNF—cytokines that play critical roles in promoting differentiation of Th1 CD4+T-cells.

Neuropeptides also promote anti-inflammatory CD4+T regulatory [Treg] cell differentiation by inducing the maturation of dendritic cells into tolerogenic APCs. Treg CD4+T-cells are autoreactive CD4+T-cells that act to inhibit the proliferation and effector functions of pro-inflammatory T-cell subsets in an antigen-specific fashion (Korn et al., 2007). In animal models and humans, decreased numbers of Treg cells are associated with the onset and relapse of autoimmune disease. *In vitro* antigen presentation by dendritic cells exposed to α-melanocyte–stimulating hormone (MSH), vasoactive intestinal peptide (VIP), or pituitary adenylate cyclase–activating peptide (PACAP) led to antigen-driven production of Treg CD4+T-cells (Gonzalez–Rey et al., 2010). Consistent with these *in vitro* observations, *in vivo* PACAP deficiency increases the incidence and severity of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (Gonzalez–Rey et al., 2010). See also EAE in Ch. 39.

Not all neuropeptides have anti-inflammatory effects. The neuropeptides in the kinin family, specifically bradykinin and substance P, have potent pro-inflammatory effects on multiple immune cells (Gonzalez–Rey et al., 2010; Carson & Lo, 2007). For example, treating myeloid cells with substance P triggers robust expression of TNF and granulocyte-recruiting chemokines as well as amplifying general responses to PAMPs. *In vivo*, these neuropeptides promote vasodilation and plasma extravasation leading to tissue edema.

However, as illustrated by the maturation stage–specific effects of 5-HT on dendritic cell function, the ultimate outcomes of neuropeptide treatment will likely rely on the combinations of receptors expressed by APCs, responding T-cells and innate immune cells. Thus the same neuropeptide may trigger very different final immune outcomes depending on the time and sites of exposure. For example, VIP inhibits macrophages and dendritic cell production of proinflammatory molecules such as prostaglandins (Gonzalez–Rey et al., 2010; Carson & Lo, 2007). However, VIP-deficient mice are resistant to EAE, while treating rats with VIP decreased the severity of experimental collagen induced arthritis (Abad et al., 2010; Deng et al., 2010). The beneficial effects of VIP in the arthritis model were associated with an increased ratio of Treg to Th17 CD4+T-cells.

IMMUNE PRIVILEGE IS NOT IMMUNE ISOLATION: THE CNS AS AN IMMUNE-ACTIVE ORGAN

From the discussions in the previous two sections, it is clear that the immune system readily interacts with the PNS. Furthermore, the immune system is able to detect and respond to self-antigens and pathogen-associated antigens located within the peripheral nervous system by the same mechanisms that apply to most tissues. However, it is also important to consider the extent that peripheral tolerance and immune defense mechanisms apply to immune surveillance of the central nervous system.

For much of the 20th century, the CNS was incorrectly considered to be isolated from the immune system (Carson et al., 2006; Schwartz & Kipnis, 2011). This view was based on experimental observations that defined the CNS as an immune-privileged site. In brief, foreign grafts placed within the parenchyma of the CNS evaded T-cell rejection and survived for more prolonged periods than similar grafts placed under the skin. The absence of any detectable T-cell rejection of foreign grafts placed in the CNS was ascribed to at least three factors:

- the absence of a population of CNS-resident immature dendritic cells able to capture antigens, migrate to draining lymph nodes and present antigen to T-cells

- the absence of a CNS lymphatic system to facilitate drainage of soluble antigens and trafficking of immune cells from the CNS to lymph nodes
- the presence of a BBB-limiting infiltration of innate immune cells, APCs and T-cells into the CNS (Chapter 1)

This inaccurate view of the CNS as an immune-isolated organ had two important but false implications (Carson et al., 2006; Schwartz & Kipnis, 2011). First, peripheral tolerance mechanisms as (detailed in the subsection Innate Immunity is triggered by evolutionarily conserved alarm signals, above) would not be able to inactivate T-cells that are autoreactive for CNS antigens. Thus, pathogen, mechanical or toxin-induced injuries to the BBB would be predicted to reveal previously hidden CNS antigens to autoreactive T-cells and to trigger CNS autoimmune disease. Second, neither the innate nor the adaptive immune systems could effectively detect infection or damage occurring within the CNS. The implications of considering the CNS as an immune-isolated organ appeared incompatible with the need to maintain a functional CNS as a condition of mammalian survival. The example of successful lifelong T-cell control of *T. gondii* infections of the CNS (see above) illustrates the fallacy of CNS immune isolation following infection; how can this be reconciled with the observed failure to reject foreign grafts within the CNS? In the following sections, we will focus on the CNS-specific regulation of innate immune cells, resident APCs and lymphocyte trafficking.

The BBB and CNS-specific regulation of leukocyte influx and efflux

The CNS is a highly vascularized organ (Carson et al., 2006; Wilson et al., 2010; Siffrin et al., 2007). The capillary network is so dense that it is estimated that all neurons and glia are within 20 microns of a capillary. Unlike other organs, the CNS is protected and sheltered from routine exposure of blood-derived products by the BBB and blood–cerebrospinal fluid (CSF) barrier. In most other tissues, fenestrated or sinusoidal porous capillaries allow variable levels of small molecules to passively move from the blood into the tissue. In addition, some molecules are actively transported into tissues by intracellular transcytosis mechanisms. By contrast, the BBB of the CNS is an organized neurovascular unit composed of astrocytes, pericytes and specialized endothelial cells lacking fenestrated structures. Tight junctions between endothelial cells of the BBB prevent paracellular transport into the CNS of blood-derived molecules. Instead, blood-derived factors must be actively transported across the endothelial barrier by transcellular mechanisms. The abluminal (CNS parenchyma) side of these endothelial cells is surrounded by basement membranes and further encased by astrocytic end-feet. Pericytes are located in the perivascular space between the endothelial cells and astrocytic end-feet. The immune function of pericytes is the subject of substantial debate. However, recent data indicate they contribute to the neurogenic potential of the neurovascular unit. It is important to note that several brain regions, referred to as the circumventricular organs (CVOs), do not have complete BBBs. Therefore CVOs provide ports of entry for passive transfer of large molecules or specific cell types into the CNS (see also in Ch. 3).

In humans, the perivascular space is termed the Virchow-Robin space and is most evident along vasculature that enters the brain from the cortical surface and is continuous with the CSF-containing subarachnoid space below the meninges (Carson et al., 2006; Wilson et al., 2010; Siffrin et al., 2007). Multiple trace studies have demonstrated that the Virchow-Robin spaces also provide a drainage route for soluble CNS antigens to passively drain to cervical lymph nodes for the purposes of promoting peripheral tolerance of CNS antigens or initiating pathogen defense responses. However, it also should be noted that this method of the antigen movement to the lymph nodes is substantially slower than that observed with the well-developed lymphatic systems present in other tissues.

To gain access to the CNS, innate and adaptive immune cells must cross either the BBB or the blood–CSF barrier in a highly regulated process (Carson et al., 2006; Wilson et al., 2010; Siffrin et al., 2007). Activated immune cells also express a variety of matrix metalloproteinases (MMPs) that facilitate migration across the BBB. Most naïve T-cells also do not express sufficiently high levels of adhesion molecules to bind the luminal (blood side) face of CNS endothelial cells and extravasate into the CNS. Consequently, entry into the CNS is largely restricted to antigen- or chemokine-activated lymphocytes with high expression of adhesion molecules such as VLA-4 and LFA-1 that bind the VCAM-1 and ICAM-1 expressed by activated endothelial cells. The dependence on these molecules is demonstrated by the effectiveness of an anti-VLA-4 therapeutic antibody (natalizumab) in the treatment of the CNS autoimmune disorder multiple sclerosis (Clifford et al., 2010). Individuals treated with natalizumab had large reductions in the numbers of T-cells trafficking into their CNS, large reductions in T-cell myelin damage and large reductions in clinical neurologic signs of CNS damage. However, the reduced T-cell trafficking came with a severe side effect associated with reduced tissue surveillance. By blocking the adhesion molecule required for efficient extravasation and subsequent infiltration into tissues, natalizumab increased the risk of JC virus, a normally benign virus, developing into a lethal brain pathogen (Clifford et al., 2010).

Leukocyte migration into the CNS parenchyma is a two-step process

Simple injury-associated disruption of the BBB is insufficient to allow substantial influx and retention of leukocytes in the CNS, in part, because the BBB can rapidly reform within hours following acute damage (Wilson et al., 2010; Siffrin et al., 2007). Leukocyte infiltration of the CNS involves more than adhesion and MMP-mediated migration across the BBB. Instead, infiltration is a two-step procedure. After extravasating from the blood, leukocytes either may remain at non-parenchymal perivascular spaces, such as observed when inflammatory infiltrates form perivascular cuffs, or may migrate into the CNS parenchyma (Müller et al., 2007; Noor et al., 2010; Reboldi et al., 2009; Ploix et al., 2010; Melchior et al., 2010). Although many factors likely regulate this process, chemokines have been demonstrated to play essential roles in each step.

Chemokine (C-C motif) Ligand 2 (CCL2) (previously referred to as monocyte-chemoattractant protein-1) is the

primary chemokine required for monocyte and macrophage entry into the CNS (Ge et al., 2009; Puntambekar et al., 2011). Genetic deletion of either CCL2 or CCR2, the receptor for CCL2, dramatically reduces the numbers of macrophages infiltrating the CNS in response to systemic immune activation or the presence of infection or other pro-inflammatory stimuli in the CNS. Different routes of entry into the CNS by different leukocyte populations may be regulated by different chemokines. Recently, the secretion of the chemokine CCL20 by the choroid plexus epithelium was demonstrated to be required for the entry of CCR6-expressing CD4+Th17 cell and the subsequent initiation of CNS autoimmunity (Kim et al., 2010; Reboldi et al., 2009). However a single chemokine may also serve to co-localize two interacting immune cell populations. CXCR3 is the receptor for C-X-C motif chemokine 10 (CXCL10). Several studies suggest that the secretion of CXCL10 by astrocytes at the blood–brain barrier acts to limit pathology during autoimmune inflammation by recruiting and retaining CXCR3+T-cells to the perivascular space. Both T-regulatory cells and effector T-cells express CXCR3. Therefore, astrocytic secretion of CXCL10 within the perivascular space promotes interaction of the anti-inflammatory CD4+Treg population with the proinflammatory effector T-cell population that it is designed to inhibit.

Additional chemokines and help from other leukocyte populations are required to support migration of lymphocytes from perivascular and periventricular regions across the glial limitans into the CNS parenchyma. For example, in a model of CNS corona virus infection, T-cells could no longer infiltrate the parenchyma in CCL2-deficient mice (Savarin et al., 2010). In the absence of CCL2-triggered influx of monocytes into the infected CNS, T-cells could not cross the glial limitans and accumulated at perivascular sites. Neither is monocyte and macrophage influx into the CNS by itself sufficient to support extravasated T-cell migration into the CNS parenchyma, even in the presence of strong pathogenic stimuli (Noor et al., 2010; Ploix et al., 2010). Instead, CNS-derived cues may be necessary. For example, neurons and glia express the T-cell chemokine CCL21 in response to infection and injury (Siffrin et al., 2007; Noor et al., 2010; Ploix et al., 2010; Biber et al., 2007). Following *T. gondii* infection, T-cells expressing CCR7 (the receptor for CCL21) migrate into the CNS along infection-induced reticular networks of CCL21. In mice deficient in CCL21, macrophages readily infiltrated the *T. gondii*–infected CNS parenchyma. However, the CCR7 expressing CD4+T-cells did not. They preferentially accumulated in perivascular spaces.

Conversely, CNS-expressed CCL21 is bioactive because it supports chemokine-driven CD4+T-cell proliferation in the cervical lymph nodes draining the CNS (Ploix et al., 2010; Aloisi & Pujol-Borrell, 2006). However, CNS expressed CCL21 was by itself insufficient to support CD4+T-cell extravasation into either the perivascular or parenchymal spaces in the CNS of uninfected mice. Furthermore, CCL21 expression leads to the spontaneous organization of inflammatory infiltrates into lymphoid-like structures in non-immune–privileged organs in response to infection or autoimmune triggers. Even in the presence of robust pro-inflammatory stimuli, CCL21 does not promote organization of infiltrating lymphocytes into lymphoid-like structures. Considered together, these studies reveal the existence of multiple levels of CNS-intrinsic regulation controlling the influx and organization of distinct populations of immune cells within the CNS.

Microglia, a CNS-specific macrophage and antigen-presenting cell

As discussed above, antigen presentation is a primary regulator of T-cell activation, necessary for retention and reactivation within tissues (Lo et al., 1999). Like all tissues, the entire CNS is populated by a tissue-specific macrophage called the microglia (Ajami et al., 2007; Ginhoux et al., 2010; Byram et al., 2004; Schmid et al., 2009). Microglia comprise between 10–20% of the total number of cells in the CNS. Morphologically, microglia display a stellate morphology in the healthy non-inflamed adult CNS, but can develop an ameboid morphology in response to pathogenic stimuli. In CNS tissue sections, it is impossible to conclusively identify a cell as a CNS-resident microglia *versus* an acutely infiltrating blood-derived macrophage because microglia express the same macrophage cell-type-specific markers as other macrophages (constitutive markers such as Iba1, CD11b, and F4/80 and inducible markers such as MHC and co-stimulatory molecules). Therefore, it is not uncommon for many studies to refer to all macrophage-like cells detected in the CNS as microglia. However, gene profiling and functional assays both indicate that CNS-resident microglia are phenotypically distinct from macrophages and dendritic cells that acutely infiltrate the CNS (Schmid et al., 2009).

Distinguishing CNS-resident microglia from CNS-infiltrating macrophages

Three methods have been used to determine the identity and origin of the myeloid cells histologically detected in the CNS (Ginhoux et al., 2010; Ajami et al., 2007). First, rodents and humans can be treated with whole-body irradiation that is just sufficient to destroy the bone marrow and its contained stem cells that routinely replace short-lived hematopoietic cells within tissues and in circulation. Unless irradiated mammals receive donor bone marrow, they will die, because they will no longer be able to generate red or white blood cells. With one prominent exception, the tissue macrophages and all hematopoetic cells found in the irradiated hosts are replaced by cells derived from donor stem cells within a few months. The only tissue macrophages that remain host derived are the parenchymal microglia that are not destroyed within the CNS. It is important to note that the perivascular myeloid cells are donor derived (Hickey & Freeman, 1988). Irradiation alters the vasculature and can facilitate inappropriate influx of myeloid cells into the CNS. However, these histologic observations have been confirmed using two additional methodologies: parabiotic linkage of the vasculature of two congenic strains of mice as well as a complex series of transgenic lineage studies. This latter study also demonstrated that microglia found in the adult CNS derive from primitive myeloid progenitors that arise before embryonic day 8 and are not bone marrow derived progenitors.

From these and similar studies, CNS-resident microglia are shown to exhibit important differences from other tissue macrophages (Carson et al., 2006; Ajami et al., 2007; Ginhoux et al., 2010; Byram et al., 2004; Schmid et al., 2009). Most tissue macrophages are short lived and continually replaced by bone

marrow–derived stem cells. By contrast, microglia are long-lived and largely self-renewing. Lineage studies further indicate that microglia arise from a primitive yolk sac hematopoietic myeloid precursor and co-differentiate as the neuroectoderm forms the CNS. Although CNS-resident microglia cannot be reliably distinguished from infiltrating macrophages by histologic methods, microglia can be identified using flow cytometric analysis of brain cell suspensions. While all other nucleated hematopoietic populations express uniformly high levels of CD45 in the mature individual, CNS-resident microglia are unique in their uniform log order lower level of CD45 expression. Recently, gene profiling studies have demonstrated that the anti-inflammatory triggering receptor expressed on myeloid cells TREM-2 is preferentially expressed on CD45low microglia, while the pro-inflammatory TREM-1 is preferentially expressed on acutely infiltrating CD45hi macrophages (Schmid et al., 2009).

Microglia are not effective at initiating antigen-driven T-cell functions

A major function of tissue macrophages, immature tissue dendritic cells and activated inflammatory macrophages is the capture of antigens within the tissue, followed by the transport and presentation of the captured antigens to T-cells located within the lymph nodes draining the tissue (Carson et al., 2006) (Figure 33-2). Grafting dye-labeled microglia and macrophages into the parenchyma of the murine CNS clearly revealed that transferred CNS-resident microglia can migrate great distances within the CNS. However, microglia could not be detected to migrate to the draining cervical lymph nodes. Indeed, up to two weeks post-transfer, nearly all of the transferred CD45low microglia are retained in the healthy murine CNS. By contrast, CD45high macrophages transferred into healthy murine CNS can be detected within the draining cervical lymph nodes within 24 hours of transfer, with few retained in the CNS. Consistent with these studies, 2-photon microscopy studies of microglia motility in response to CNS injury demonstrated rapid motility of microglial process, but did not report rapid migration of microglial cell bodies away from the injured area. These data indicate that microglia do not present tissue antigens to lymph node at the same rate (if at all) as other tissue macrophages or immature dendritic cells.

Several studies have used irradiation chimeras to selectively express MHC expression on CNS-resident microglia versus peripheral immune cells. Consistent with their failure to emigrate from the CNS to lymph nodes, these types of studies have confirmed that CNS microglia are relatively ineffective at initiating antigen-driven CD4+T-cell responses (Carson et al., 2006; Schwartz & Kipnis, 2011; Dilger & Johnson, 2008; Byram et al., 2004). Instead, this function is provided primarily by infiltrating dendritic cells and to a lesser extent by perivascular macrophages. By themselves, these data do not indicate that microglial antigen presentation is irrelevant. Additional studies suggest that antigen presentation may have purposes complementary to professional APCs (Carson et al., 2006; Schwartz & Kipnis, 2011; Byram et al., 2004). For example, CD4+T-cells can develop neuroprotective functions able to slow the rate of axotomy-induced cell death of facial motoneurons located in the brain stem (Byram et al., 2004). Using irradiation chimeric models and adoptive transfer of axotomy-activated CD4+T-cells, microglia were again demonstrated to be unable to initiate axotomy-induced CD4+T-cell responses. However, MHC class II–expressing microglia were demonstrated to be absolutely essential to evoke or sustain the neuroprotective functions of CD4+T-cells initially activated by the peripheral immune system. In addition, several studies have reported that depending on the T-cell activation state, microglial-T-cell interactions can promote microglia to acquire either growth factor–producing, neurogenic, neuro-repairing phenotypes or cytotoxic, neurodestructive phenotypes.

Taken together, these studies illustrate that two functionally distinct APC populations serve the CNS: a long-lived and/or self-renewing population of CD45low CNS-resident microglia (the majority of macrophage-like cells in the CNS) and a short-lived population of macrophages and APCs that are readily replaced by bone marrow.

The CNS microenvironment actively regulates the phenotype of microglia and infiltrating immune cells

Gene profiling studies have demonstrated that migration of macrophages into the CNS alters their gene expression patterns, and even induces macrophage expression of microglial molecules rarely expressed outside the CNS (Schmid et al., 2009). For example, macrophages infiltrating the CNS during LPS-induced inflammation or during the early remission phases of EAE begin to express the tolerance-related transcript Tmem176b. Experimental expression of Tmem176b inhibits acquisition of classical APC functions as well as increasing the rate of apoptosis of activated microglia and macrophages. These and similar data demonstrate the robust and active regulatory role of the CNS microenvironment and raise an unresolved and highly debated issue in neuroimmunology. Can CNS-infiltrating macrophages ever acquire partial or full microglial phenotypes following CNS pathology associated with robust immune cell infiltration? Specifically, can blood-derived macrophages decrease CD45 expression to microglial levels and acquire microglial-specific APC functions.

As yet, the mechanisms by which the CNS regulates immune cell function are still being identified (Dilger & Johnson, 2008; Biber et al., 2007). Neurons express a large number of molecules that inhibit and or redirect microglia and macrophage activation. For example, fractalkine, CD200 and CD22 are all expressed on the surface of CNS neurons, while their receptors are expressed on unactivated and activated microglia and macrophages. Deletion of these molecules or their receptors on microglia and macrophages primes both microglia and macrophages to develop classical activation states more rapidly than wild-type cells and in response to lower doses of pro-inflammatory stimuli.

The CD200 receptor is expressed at similar levels on both microglia and macrophages (Biber et al., 2007). Thus neuronal regulation of both microglia and macrophages activation can be coordinately regulated via this pathway. By contrast, CX3CR1, the fractalkine receptor, is expressed at higher levels on microglia, while CD45, the receptor for CD22, is expressed at higher levels on CNS-infiltrating macrophages (Lo et al., 1999; Carson et al., 2006; Biber et al., 2007). Thus, neurons can simultaneously regulate microglia and macrophage functions using cell

type–specific mechanisms. Lastly, as discussed for macrophages in above, microglial functions are also regulated by neurotransmitter exposure. For example, glutamate amplifies classical activation responses while norepinephrine reduces these responses. These types of data demonstrate the potential for the types and degree of neuronal activity to regulate immune responses of CNS-resident microglia and CNS-infiltrating immune cells (Carson et al., 2006; Carson & Lo, 2007; Biber et al., 2007).

IMMUNE-REGULATED CHANGES IN NEURONAL FUNCTION AND MAMMALIAN BEHAVIOR

The previous sections have described multiple neuroimmune interactions. When these interactions are dysregulated or inappropriately prolonged, they can be maladaptive for CNS function. Notably, proinflammatory cytokines and reactive oxygen species produced by activated innate and adaptive immune cells have demonstrated potential to damage neurons and glia (Dilger & Johnson, 2008; Biber et al., 2007). Thus, cytotoxic immune functions are well recognized to contribute to the pathogenesis of autoimmune and neurodegenerative disorders. More recently, profiling studies have begun to demonstrate aberrant expression of immune-associated molecules in tissue taken from humans with classical neurologic disorders such as schizophrenia and autism (Dilger & Johnson, 2008; Biber et al., 2007) (see in Ch. 59). The extent to which CNS immune responses are contributing to—or are merely responding to—CNS dysfunction is unknown. Nor do we know all of the mechanisms by which immune cells can regulate CNS function. However, in animal models, T-cell contact with neuronal axons and T-cell–produced granzyme (apoptosis inducing serine protease) have been implicated in the initiation and progression of some forms of seizure activity. Prolonged purigenic and/or cytokine activation of microglia and macrophages can alter pain thresholds. Conversely, inappropriate initiation or prolongation of immune-mediated neurorepair functions can promote tumor growth or prevent elimination of neurons and glia that should be eliminated by programmed death mechanisms.

Neuroimmune interactions can also induce patterns of behavior, referred to as sickness behavior, that are unpleasant for an individual but that have adaptive survival consequences (Dilger & Johnson, 2008). During inflammatory responses to pathogens, systemic production of TNF, IL-1 and IL-6 acts on the CNS vasculature to promote glial production of proinflammatory cytokines and prostaglandins within the CNS. These cytokines also act on the vagal nerve and may be actively transported across the BBB into the CNS. This in turn initiates a program of behaviors that serve to enhance immune attack on the pathogen and survival of the individual. Specifically, systemic inflammation promotes

- fever, generating a non-optimal environment for pathogen survival

ONE PATHOGEN BUT THREE IMMUNE RESPONSES WITH THREE NEUROLOGIC OUTCOMES

Monica J. Carson

Measles is a highly contagious illness caused by paramyxovirus family virus. Almost all non-immune children contract this respiratory disease if exposed to the virus. While immunization effectively controls the incidence of measles, decreasing rates of immunization in developed regions and lack of access to immunization are allowing the immunologic side effects of measles to emerge as a significant health risk. Here we present three rare complications of measles infection to demonstrate the consequences of too much, too little or just the wrong type of neuroimmune responses.

1. An immune response sufficiently effective to clear a pathogen can also cause lethal brain damage after the pathogen has been effectively cleared. For example, acute post-infectious measles encephalomyelitis (APME) is characterized by abrupt onset of fever, seizures and multifocal neurological signs one to two weeks after appearance of the measles rash and is fatal in 10–20% of patients. Despite the absence of detectable measles virus in the CNS of these patients, clinical features of APME correlate with robust influx of blood-derived macrophages and lymphocytes into the CNS. The sum of the published data suggest that during immune-mediated clearance of measles from the body, pathogen-provided alarm signals (PAMPs) led to the inappropriate activation of T-cells able to recognize self-antigens in 0.1% of patients infected with measles.

2. Ineffective immune responses are ineffective in removing PAMP signals and thus promote chronic neurotoxic inflammation. Subacute sclerosing panencephalitis (SSPE) is a very rare complication of measles infection and is associated with continual low-grade inflammation of the CNS. Clinical symptoms emerge 5–10 years after systemic clearance of the measles virus and include mental deterioration, appearance of myoclonus, optic atrophy and akinetic mutism. Based on experimental models, SSPE is presumed to result from CNS infection by partially defective measles virus particles incapable of budding from the surface of infected cells. The budding-defective viral clones are able to persist and spread throughout the CNS because of ineffective antigen presentation and detection of viral particles. Clinical pathology is likely the combined result of persistent production of TNF and IL-1β, accumulation of intrathecal oligoclonal IgG, as well as the persistence of measles virus within cells of the CNS.

3. Too little immunity can result in normally benign viruses, bacteria and parasites lethally disrupting brain function. In this situation, brain damage and/or death can be due to competition for metabolic components, to disruption of CNS intracellular signaling by pathogen products or to direct pathogen killing of CNS cells. Measles-infected children with viral or drug-induced immunodeficiencies cannot always control the viral infection, resulting in a general progressive neurological deterioration that includes seizures. Neuronal damage, astrogliosis and inclusion bodies in both neurons and glia are observed in brain pathology samples from infected individuals. The only effective treatment in this situation is to attempt to halt viral replication with antiviral drug therapies.

- anorexia, which limits systemic availability of nutrients and cofactors
- lethargy and slow-wave sleep, which serve to conserve the individual's metabolic resources
- decreased social, reproductive and exploratory behaviors

Sickness behavior is usually transient, disappearing with immune-mediated clearance of pathogen and the subsequent resolution of systemic immune responses (Dilger & Johnson, 2008). However, recent studies suggest that sickness behavior may facilitate the onset or relapse of clinical depression. It is speculated that susceptibility may result from hyperproduction of TNF, IL1 or IL6, hypoproduction of IL-10, or less-efficient serotonergic function as occurs with homozygosity for the short allele of the 5-HT transporter gene.

SUMMARY: MANIPULATING NEUROIMMUNE INTERACTIONS

This chapter has detailed the cross-regulatory interactions occurring between the immune and nervous systems. Maturation of the nervous system is in part experience driven. Therefore, age, environmental factors and neuronal activity may alter neuroimmune interactions with detrimental consequences for neuronal function. Thus, it is tempting to suggest that inhibiting neuroimmune responses is the correct therapeutic choice for neurodegenerative disease. However, the better choice might be to manipulate neuroimmune interactions to redirect immune-cell functions. For example, autoreactive T-cells can provide neuroprotection both directly and indirectly by stimulating CNS production of growth and survival factors (Schwartz & Kipnis, 2011; Dilger & Johnson, 2008). Similarly, increasing influx of macrophages into the CNS and decreasing neuronal inhibition of microglial phagocytic activity are both effective measures to decrease amyloid burden in murine models of Alzheimer's disease.

Continued dissection of the molecular basis by which neuroimmune interactions are regulated has the promise of yielding promising immunotherapies for neurodegenerative and other classic neurologic diseases.

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