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Review

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Get It through Your Thick Head: Emerging Principles in Neuroimmunology and Neurovirology Redefine Central Nervous System "Immune Privilege"

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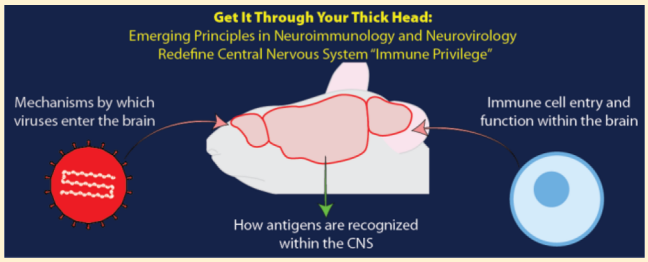
ABSTRACT: The central nervous system (CNS) coordinates all aspects of life, autonomic and sentient, though how it has evolved to contend with pathogenic infections remains, to a great degree, a mystery. The skull and cerebrospinal fluid (CSF) provide protection from blunt force contacts, and it was once thought that the blood-brain barrier (BBB) was a fortress that restricted pathogen entry and limited inflammation. Recent studies, however, have caused a revision of this viewpoint: the CNS is monitored by blood-borne lymphocytes, but can use alternative strategies to prevent or resolve many pathogenic challenges. In this Review, we discuss emerging principles that indicate how the CNS is immunologically unique from peripheral tissues. We focus on developments that include glymphatics, recently characterized brain lymphatic vessels, distinctions in innate and adaptive immune strategies, novel points of entry for neurotropic viruses, and, finally, how the periphery can influence CNS homeostasis and immune responses within the brain. Collectively, these attributes demand a re-evaluation of immunity in the brain: not privileged, but distinct.



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For decades, the magnitude of the host response within the central nervous system (CNS) was considered to be less robust than that in non-CNS tissues. That is, introduction of antigens into the brain elicited a less potent inflammatory response. This concept was termed "immune privilege", and implied that the brain had adapted to be protected from the potentially deleterious consequences of immune action. This notion also provided an easy explanation for many immune-based CNS diseases: pathogenesis ensued when immune cells breached the otherwise immune-privileged brain.

Over the past few years, our view of CNS immunity has radically matured, and most researchers in the field now feel that the term "immune privilege" is misleading. Generally, immune privilege was defined as the ability of a tissue or organ to tolerate an introduction of antigens without a consequent inflammatory response. We now know, however, that lymphocytes circulate through the healthy brain, immune responses can occur in the parenchyma without lasting consequence, and extensive cross-talk between the brain and the periphery exists. In this Review, we discuss some of the recent shifts in our understanding of basic principles of neuroimmunology, chiefly focused on the neurovirology literature. We aim to provide an overview of immune action within the virus-infected CNS, and discuss how peripheral events can shape such infections and resultant neuropathogenesis.

THE SKULL IS BOTH A PROTECTOR AND A LIABILITY

The skull developed in parallel to the specialization of the notochord and the emergence of Craniata.[1] Prior to this development, the primitive brain was the sensory organ, integrating input from the environment and coordinating an appropriate response. Of necessity, it was adjacent to the primary sensing organs, typically found at the head/front of the organism. As vertebrates evolved, a protective shell encased the brain, shielding it from direct injury. The spherelike nature of the bones that comprise the skull helped to deflect and distribute blunt force assaults; the brain is the only organ that benefits from virtually complete bone-based security. Moreover, the spinal cord is surrounded by a spiky armor of vertebrae and cushioned by cerebrospinal fluid (CSF), further protecting the CNS from injury. In the late 1800s, scientists, including Nobel Laureate Paul Ehrlich, began to realize that the brain was protected not only mechanically by bone and fluid, but also at the cellular level by the blood-brain barrier. The unique functions of this cellular barrier were appreciated when Edwin

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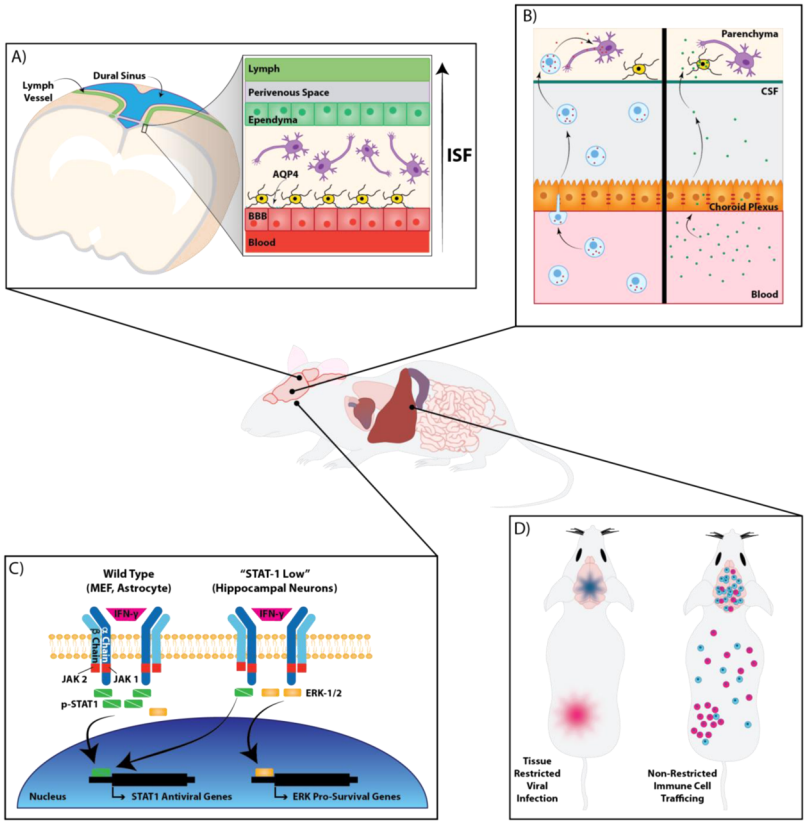

Figure 1. Recent changes in neuroimmunology. (A) Lymph vessels in the brain that line the dural sinuses. (Inset) Movement of interstitial fluid from blood through aquaporin 4 (AQP4) channels on astrocytes lining the brain vasculature, through the interstitial space of the parenchyma, collecting in the perivenous space before draining through lymph vasculature. (B) Viral entry through the choroid plexus. (Left) Infected cells may ferry virus (red) through the CP into the CSF and migrate into the parenchyma to infect cells such as neurons (purple) or astrocytes (yellow). (Right) Alternatively, virus in circulation (green) may infect choroid plexus cells directly, leading to viral release into the CSF and parenchyma where it can access permissive cells of the CNS. (C) Differences in the cytosolic availability of key signal transduction molecules (e.g., STAT1) result in distinct cellular responses between primary mouse hippocampal neurons and other cell populations. (D) Despite the tissues restriction of various viral infections (e.g., CNS-tropic (blue) and viscerotropic (pink)), the immune responses generated to these challenges are not tissue-restricted, and activated immune cells generated to either infection may traffic to either location. Unique immunopathological diseases may result. Note that all schematics are drawn to highlight basic principles, and are not intended to accurately portray neuroanatomy.

Goldmann, a student of Ehrlich's, used trypan blue dye, which normally perfuses all tissues when injected into the blood, but which was selectively excluded from the CNS.[2,3]

However, the skull that affords protection from blunt injury also imposes a constraint during inflammatory processes: the inflamed brain has limited space for expansion, unlike the swelling that can be transiently tolerated in many peripheral tissues. Anatomical and cellular barriers limit entry of immune cells that may cause edema or even direct damage to (generally) nonrenewable CNS neurons. These observations further contributed to the concept of immune privilege: that entry of blood borne lymphocytes and their mediators into the brain parenchyma was restricted.

GLYMPHATICS AND THE BRAIN'S LYMPHATIC SYSTEM

One of the chief tenets supporting the immune privileged nature of the brain was the apparent absence of a functional lymphatic system, present in virtually all other organs. This system transports interstitial fluid and waste products out of tissues and into the blood, where they are targeted for removal. In infections, such transport is essential for delivery of antigens and professional antigen presenting cells to lymph nodes for education of the adaptive immune response. In peripheral organs, this clearance process is governed by osmotic pressure, and its importance becomes clear when lymphatic flow is blocked, leading to massive fluid accumulation (edema).

Recently, evidence has emerged that there is extensive CSF and interstitial fluid (ISF) exchange throughout the brain, a process termed "glymphatics" by Maiken Nedergaard and her colleagues to underscore the similarity to the lymphatic system, and to acknowledge the critical contribution of glia in this process. These studies, among others, showed that a pathway of waste removal does exist within the brain, facilitated by CSF entering the brain parenchyma and spinal cord via aquaporin 4 water channels on astrocytes that surround the brain vasculature; genetic ablation of aquaporin 4 substantially reduced clearance of interstitial solutes. This wave of CSF entry drives ISF, which carries with it extracellular proteins and solutes, toward the perivenous space where it collects and drains toward the cervical lymph nodes (Figure 1a).[4−7] The CSF−ISF exchange results from both arterial pressure and vasomotion to move the fluid through the parenchyma, in essence, flushing extracellular debris from the parenchyma, including beta amyloid, a protein that contributes to Alzheimer's disease. This clearance of interstitial proteins and solutes may also include pathogenic antigens that may be present in the CNS at the time of an infection. It is intriguing

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that this process is accelerated during resting phases, such as sleep,[7,8] which some have contended is a key evolutionary basis for why sleep exists. As noted in an NIH news release that accompanied publication of the article, "a good night's rest may literally clear the mind."[8,9] Historically, scientists were aware that lymphatic draining did occur in the CNS by observing the migration of fluorescent tracer dye injected into gray matter draining out to the cervical lymph nodes.[10] These important observations, now viewed in light of recent findings, highlight the connection between the glymphatic system and a lymphatic system in the brain.

Since this discovery of a surrogate lymphatic system in the brain, two studies published in 2015 showed that in addition to the glymphatics system noted above, a bona fide lymphatic vasculature line the dural sinuses and meningeal arteries (Figure 1a). These lymphatic vessels not only drain ISF but also permit leukocytes to migrate from the CNS to the draining cervical lymph nodes.[11,12] The lymphatic vasculature is associated with the meningeal layer and lines the dural sinus along the crest of the brain following a path that drains out of the skull, along with arteries and cranial nerves, through the foramen at the base of the skull.[11,12] These vessels possess attributes of classical lymphatic endothelial cells, and are connected to the deep cervical lymph nodes.

The presence of these drainage systems within the CNS is evidence that there is a constant flow and exchange of proteins within the brain and the blood. As debris is taken out of the brain and deposited into the blood for removal, it affords the immune response an opportunity to survey proteins produced in, or by, resident brain cells. One could speculate that if such proteins belong to pathogens or are otherwise-sequestered self-proteins, a host response may be initiated. The notion of constitutive surveillance dovetails with the observation that T cells are readily detectable in the CSF of healthy individuals ($1.0-3.0 \times 10^3$ cells per mL).[13] The T cells found in healthy CSF are primarily $CD4^+$ central and effector memory T cells, as opposed to naïve T cells,[13,14] allowing for a rapid recall response to an antigenic encounter within the CNS. Additionally, resident memory $CD8^+$ T cells persist in the brain following an acute viral infection. These $CD8^+$ T cells exhibit a molecular signature that is distinct from circulating memory T cells.[15,16] They are characterized by expression of CD103, an integrin whose expression is dependent on recognition of cognate antigen within the infected tissue. Moreover, CNS resident memory T cells do not depend on the same survival signals as circulating memory T cells. When isolated from the brain, $CD103^+$ memory $CD8^+$ T cells did not survive when cultured with IL7- or IL15-supplemented media, whereas isolated circulating memory $CD8^+$ T cells did survive.[15,16]

VIRAL INVASION INTO THE BRAIN

A surprisingly large number of pathogens, including many viruses, can infect the CNS, though it is generally believed that CNS invasion is a rare event. The rarity of pathogenic infection of the CNS fueled the idea that the brain is sequestered from pathogens that circulate peripherally. We now know that the blood-brain barrier is not an impenetrable shield to entry of pathogens. Neurotropic viruses include those that are well-known CNS pathogens (e.g., rabies, polio, and many herpesviruses), as well as less-appreciated neurotropic viruses, including influenza, West Nile virus, and measles virus. Historically, it was assumed that there were two primary ways by which viruses, reproducing in the periphery, gained access to the CNS: via transneuronal migration, and as stowaways in circulating blood cells. Surely the best example of a virus that gains access to the brain via trans-synaptic spread are the herpesviruses, including herpes simplex viruses I and II. These viruses encode viral proteins that utilize the host cell machinery to migrate within the axon on microtubules via retrograde transport using dynein motors or anterograde-directed transport with kinesin motors. Once the virus gains access to CNS neurons, virions can be released to infect adjacent or innervated cells.[17] Polio has also been shown to use neuronal pathways, as axotomy (experimental axon resection) limits polio entry into the brain.[18]

Viruses can also enter the CNS by "hiding" within the blood-borne lymphocytes that we now know patrol the brain (Figure 1b). Human immunodeficiency virus-infected $CD4^+$ T cells and monocytes are ferried into the parenchyma, where virus particles can then be released. In a similar way, JC virus, a polyomavirus, is brought to the brain either in infected B cells[19] that cross the blood-brain barrier, as free virus, and/or by infecting endothelial cells that comprise the barrier.[20] For neurotropic viruses, invasion of the parenchyma is considered a one-way trip: CNS entry is presumably a dead-end for the virus, and unless the infected brains are exposed (or eaten, as true for transmissible spongiform encephalopathies), the virus has no way back out of the brain. Given the detection of lymphatics that underscore greater cross-talk between the blood and the brain, it is possible that viruses that reproduce in the CNS could be transported back out into the periphery in much the same way that they first gained access.

In addition to these known ports of entry into the brain, it has recently been appreciated that the choroid plexus is also a gateway for pathogens into the CNS (Figure 1b).[2,12] The choroid plexus is comprised of epithelial cells that produce cerebrospinal fluid. These epithelial cells are connected by tight junctions and regulate transmission of substances from the blood to the CSF. The entry of immune cells, as well as pathogens, into the CSF through the choroid plexus has been discussed in a recent review,[2] in which the exploitation of the choroid plexus by bacterial, parasitic, and viral pathogens is described. Mouse models of enterovirus infection, which include coxsackievirus, were used to show this unique mode of viral entry into the brain. Coxsackievirus crosses the choroid plexus by infecting myeloid cells in the periphery, which are then transported through the choroid plexus into the CSF. This is in contrast to Echovirus (another enterovirus), which directly infects the epithelial choroid plexus cells and releases new virus particles into the CSF (Figure 1b).[2,21]

Choroid plexus cells detect infection via pattern recognition receptors including Toll-like receptors, triggering an innate immune response.[22,23] This response results in the induction of various adhesion molecules, including ICAM1 and VCAM1, as well as various cytokines and chemokines such as TNFα, IL-6, IL-8, IL-1β, CCL5, and CXCL1−3.[21,22] These proinflammatory cytokines and recruitment signals aid in the recruitment of immune cells from the periphery, and result in their transmigration through the choroid plexus into the infected CNS, contributing to virus-induced neuroinflammation.

TYPE I AND TYPE II MECHANISMS OF NEURONS RESPONDING TO DIRECT VIRAL INFECTION

While all cell types are morphologically and physiologically distinct, certain aspects of CNS neurons make them particularly unique. Although the detection of neural stem cells throughout

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life has indicated that some neuronal populations may be replaced, in general, neurons of the CNS are nonrenewable. This is of direct relevance to infections and resultant inflammation, as loss of these chiefly irreplaceable cells could be catastrophic.

The CNS uses many of the same tools and approaches as peripheral organs to respond to a pathogenic challenge, yet does so in a way that may promote neuronal survival. The canonical response to a viral infection is primarily cytolytic, mediated via direct target cell lysis by cytotoxic T cells and cytokines. However, as noted above, such strategies would pose a clear danger if deployed in the brain. Thus, the immune response may be sufficiently different in the CNS to prevent the loss of nonrenewable cells as well accounting for limited space to accommodate the swelling/edema that often accompanies inflammation. Interestingly, it is the neuronal response to these cytokines, rather than different immune players, that appears to afford neuronal protection. Two examples follow.

An early line of innate defense is the induction of type I interferons (IFN). Once a cell is alerted to viral infection, usually through engagement of pattern recognition receptors, it will produce and respond to type I IFNs, IFNα and IFNβ, resulting in a signaling cascade that culminates in the synthesis of potent antiviral gene products.[24−26] This response, however, is not invariant among all cell types: cell populations, including neurons, can differ in their response to these potent mediators.[26−28] Primary mouse hippocampal neurons express higher homeostatic levels of type I IFN compared to readily renewable cells such as mouse embryonic fibroblasts (MEFs), which may allow for a more immediate and robust response following infection. Surprisingly, however, STAT1 and STAT2 signaling kinetics are delayed in these neurons, resulting in reduced expression of classical antiviral interferon-stimulated genes. These data have led some to propose that alternative signaling pathways are used downstream of neuronal type I interferon engagement. Alternatively, if infection does occur, the type I IFN response may be muted to protect neurons from the potentially cytotoxic outcome of induction of a robust antiviral program.[29]

The kinetics of type II IFN responses in neurons are also unique. Primary mouse hippocampal neurons show a delayed and muted transcriptional response to IFNγ stimulation compared to MEFs; this is attributable, at least in part, to reduced STAT1 bioavailability, delayed STAT1 phosphorylation and activation, and extended receptor protein phosphorylation of JAK1/2 cytoplasmic subunits.[30−32] For some viral infections in the CNS, including measles virus,[33] IFNγ is critical for virus control and survival: without IFNγ, infected mice die. Interestingly, although IFNγ can be cytotoxic to replicating cell types,[34,35] it is not toxic to hippocampal neurons in culture, again implicating alternate pathways downstream of IFNγ binding (Figure 1c). This has also been observed in vivo, in which IFNγ mediates clearance of Sindbis virus via a noncytolytic mechanism.[28,36,37] These data suggest that altered interferon kinetics may be one mechanism by which certain neurons are protected from the otherwise toxic consequences of interferon engagement and signaling.

In addition, there is evidence that IFNγ is actually neuroprotective. When hippocampal neurons are exposed to staurosporine in vitro they undergo apoptosis, as determined by detection of pyknosis (nuclear condensation) and cleaved caspase 3. In contrast, when neurons are cultured in media containing staurosporine and IFNγ, there is a significant decrease in the number of cells undergoing apoptosis.[38] Thus, it is not the absence of crucial cytokines such as the interferons in the brain, but rather differences in how these signals are interpreted by resident CNS cells, that dictates the unique outcome within the brain (Figure 1c).

CD8$^+$ T cells have a repertoire of mechanisms they can use to resolve a viral infection including TRAIL, Fas ligand, and perforin/granzyme in addition to their secretion of IFNγ. While cytokines such as IFNγ are critical for some experimental infections, these other pathways may be operative for others. For example, in the context of a WNV infection in the CNS, IFNγ is critical for early control of infection,[39] whereas perforin, FasL and TRAIL mechanisms are required for survival.[40−42]

Although viral control in the absence of appreciable neuronal death would seem to be an ideal solution to neurotropic infections, there may be a long-term consequence to this strategy. The terms "clearance" or "resolution" are often used as a surrogate when an alleviation of symptoms in mice is observed. However, mouse survival does not necessarily imply complete clearance of the virus: viral replication may be suppressed to subacute levels, rather than reflect a complete elimination of viral genomes and proteins. This, of course, is known to occur for neurotropic DNA viruses such as the herpesviruses, which can establish latency in neurons of the peripheral nervous system. Latency is accompanied by restricted gene expression and circularization of the viral genome into an episome, perhaps to minimize endonucleases.[43]

Recent studies have shown that neurotropic RNA viruses may also "persist", in some form, in the CNS. Viral RNAs have been detected long after apparent resolution of a number of neurotropic infections, including Sindbis and rabies virus.[44,45] The presence of these viral RNAs were generally dismissed as viral fossils, and not likely to have long-term consequences for the mouse.[46,47] However, at least for some of these viruses, RNAs may be reactivated to result in transcription and translation of viral mRNAs and proteins, especially under conditions in which the murine host is immunosuppressed.[15,16,48]

PART OF A WHOLE: THE EFFECTS OF PERIPHERAL IMMUNE RESPONSES ON CNS HOMEOSTASIS

For approximately a decade, it has been appreciated that activated T cells, specifically CD8$^+$ T cells, traffic to nonlymphoid tissues regardless of their origin or site of activation, presumably to provide holistic protection in the event of pathogen dissemination.[49−51] This raises an interesting point: if the host is challenged with multiple, tissue-restricted, nonoverlapping antigenic encounters (e.g., a respiratory tract-restricted virus and a gastrointestinal tract-restricted bacteria), do the T cells activated in response to one pathogen migrate nondiscriminately to a tissue in which no antigen is apparent, and, if so, can this result in unique forms of tissue damage? This is especially relevant for those who use mice as models for humans. Unlike laboratory mice that live a relatively pathogen-free existence, humans are subjected to a diverse barrage of antigens—viruses, bacterial infections, allergens, and vaccines, among others. While some fascinating studies have emerged to explore the direct influence of one pathogen on another (e.g., how infection with one pathogen may influence the life cycle of another), we are only beginning to appreciate that the host responses to these infections may also interact.

The subfield of heterologous immunity studies the beneficial and detrimental effects of T cells that engage peptides from

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distinct pathogens. Such cross-reactivity has potential beneficial consequences. For example, heterologous immunity may afford increased protection in the event of exposure to the second pathogen, but can also be detrimental if this cross-reactivity impedes the control of both infections and/or triggers autoimmunity (to cross-reacting self-peptides, for example). How heterologous immune responses are generated, and the consequences of these responses in polymicrobial settings, are discussed in an excellent review[52] as well as in a recent research report in which memory $CD8^+$ T cell responses were found to be altered when elicited in a coinfection setting using lymphocytic choriomeningitis virus (LCMV) and Pichinde virus. When these two viruses elicited "competing" immune responses, the result was decreased protective immunity with enhanced pathology following a rechallenge with one of the viruses.[53]

Studies have also shown that T cells can mediate pathogenesis independently of antigen cross-reactivity. For example, $CD8^+$ T cells activated in response to a LCMV infection exacerbate *Leishmania major* infection, in contrast to the prediction that LCMV-elicited $CD8^+$ T cells would afford increased protection in leishmaniasis due to their strong IFNγ response. In this system, LCMV-activated $CD8^+$ T cells infiltrating leishmanial lesions caused increased pathology via a granzyme B dependent mechanism. Blocking NKG2D, a receptor found on both natural killer cells as well as $CD8^+$ T cells,[54,55] resulted in a reversal of pathology.[56]

The CNS may be particularly vulnerable to diseases that result from polymicrobial challenges. Earlier, we noted that the brain is routinely patrolled by blood-borne lymphocytes and that antigens within the brain can be carried, through the lymphatics, into the periphery. These concepts, coupled with the risk of temporally overlapping immune responses to cause detrimental consequences, may be particularly worrisome in the CNS, where the immune response must be uniquely controlled. Many CNS diseases of unknown origin contain an inflammatory component as part of their pathology, yet the direct cause of these human diseases (e.g., amyotrophic lateral sclerosis, multiple sclerosis[57−60]) remains to be determined. That said, the potential for "peripheral" immune responses to enter the CNS and cause neuropathology, perhaps relevant to human neuroinflammatory diseases of unknown etiology, should not be excluded.

Work from our laboratory took advantage of a novel disease model to explore the consequences of temporally overlapping immune responses to two distinct viruses. LCMV is a member of the arenavirus family, and, when given by an intraperitoneal route, is restricted to tissues in the periphery, including the kidneys, spleen and liver. Infection by this route results in viral resolution and mouse survival. Similarly, measles virus infection of transgenic mice that express a neuron-restricted measles virus receptor also results in a host response that resolves the infection in the absence of morbidity or mortality. When these infections are combined, however, the outcome is severe CNS pathology and death in the majority of mice, due to $CD8^+$ T cell mediated destruction of the ependymal lining of the ventricles, CSF leakage into the brain, and resultant edema. Thus, the presence of an immune response to a peripherally restricted viral infection influences the outcome of a second, typically nonpathogenic, infection within the CNS (Figure 1d).[61]

The peripheral immune response not only alters the nature of a CNS targeted immune response but can also result in "sterile inflammation" independent of direct infection. Utilizing both acute and chronic LPS-induced inflammation models, detectable increases in IFN-stimulated genes were noted in the CNS 48 h after induction of a peripheral immune response.[62] This long distance "preparation" and upregulation of IFN-stimulated genes can occur in the context of viral infections that are restricted to the nasal mucosa yet, but that, nevertheless, can upregulate antiviral gene transcription deep within the brain.[63] This implies that the CNS can sense, and respond to, events in the periphery. Consequently, challenges in non-neuronal tissues may nevertheless affect gene expression and response within the brain, including the impact of commensal microbes on CNS homeostasis,[64−67] surely a topic that deserves more attention as studies on the microbiome progress.

■ ONWARD

The past few years have been a notably productive period for the fields of neurovirology and neuroimmunology. We are not only learning more about individual virus-neuron and immune cell-neuron interactions, but are uncovering new principles that govern how infections are contained in the context of the CNS. New data identifying the lymphatic system within the CNS, the implications of glymphatics for brain homeostasis, and the detection of subacute viral infections within the CNS, all suggest that there is much left to discover about immunity in the brain.

A broader conclusion from these studies is that the brain is not a satellite of the body, isolated from the effects of the host immune response, but rather is actively monitored by, and accessible to, blood-borne lymphocytes and their mediators. Moreover, unlike the rare (and often fatal) consequences of acute viral challenges in the brain, the potential that viruses may establish long-term quiescent infections, perhaps to be reactivated later in life, broadens our consideration of the pathogenic potential of neurotropic viruses, and how the host response restricts these threats.

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■ KEYWORDS AND CONCEPTS

Immune privilege, a tissue environment that is shielded from the inflammatory immune response; glymphatics, a lymph-like system of interstitial fluid exchange in the CNS that is dependent on glial cells; interferons, a group of soluble proteins that stimulate transcription of antiviral genes in response to

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pathogen detection; virus, an obligatory intracellular parasite comprised of protein and nucleic acid; polymicrobial infection, infection with more than one pathogenic challenge

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