◆ REVIEW ARTICLE ◆

Intracerebral regulation of immune responses

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Major progress has been made over the last years in our understanding of the mechanisms underlying immune privilege and immune surveillance of the central nervous system (CNS). Once considered a passive process relying only on physical barriers, immune privilege is now viewed as a more complex phenomenon, which involves active regulation of immune reactivity by the CNS microenvironment. Evidence has also emerged that the immune system continuously and effectively patrols the CNS and that dysregulated immune responses against CNS-associated (exogenous or self) antigens are involved in the pathogenesis of various neurological diseases. In this article we shall briefly review current knowledge of how the immune response is regulated locally in the CNS and which cell types and molecular mechanisms are involved in shaping intracerebral immune responses.

Keywords: antigen presentation; astrocytes; central nervous system; chemokines; cytokines; dendritic cells; inflammation; microglia.

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Introduction

The central nervous system (CNS) has been shown to be an immune-privileged site where certain viruses and bacteria can persist without sensitizing the immune system and transplanted allogeneic tissue can survive for a prolonged period of time. For many years, this was thought to result from the lack of lymphatic drainage and dendritic cells and the presence of blood–brain and blood–cerebrospinal fluid (CSF) barriers that prevent the afferent and efferent arms of an immune response to intracerebral antigen from occurring. It is now known that immune privilege in the CNS is a much more complex process that employs multiple mechanisms to avoid unnecessary immune activation and to protect neurones and glial cells from damaging inflammation (1). Evidence is growing that the CNS environment itself plays a role in inhibiting the activation of immune cells, both those residing within the neural tissue and those recruited from the periphery. This is thought to be accomplished by local production of immunosuppressive cytokines (eg, transforming growth factor-β (TGF-β)) and neuropeptides (eg, vasoactive intestinal peptide, α-melanocyte stimulating hormone) (1) and by intracerebral expression of Fas ligand, which may promote apoptosis of invading Fas^+ immune cells during CNS infection and autoimmunity (2). Neurones themselves, through direct cell-to-cell interactions (3) and secreted factors such as neurotrophins and neurotransmitters (4), have been proposed to play a major role in down-regulating the activation state of CNS intrinsic macrophages, the microglia. Immune privilege in the brain is also thought to be actively maintained through immune deviation mechanisms that involve preferential induction of humoral responses to CNS-derived antigens and supression of CNS antigen-specific delayed-type hypersensitivity (1, 5).

During the past two decades, there has also been considerable improvement in our understanding of the mechanisms that ensure effective immune surveillance of the CNS. This field of research has been mainly stimulated by the need to get more insight into the role of immune responses in CNS autoimmune diseases, such as multiple sclerosis and paraneoplastic neurological disorders, and in infectious or post-infectious encephalopathies. Extensive research carried out in animal models has unravelled that the CNS is not totally secluded by the peripheral immune system and that a well-developed network of innate immune cells surveys all interfaces between the CNS and the periphery in addition to the neural parenchyma itself. Major advances in our understanding of the mechanisms involved in CNS immune surveillance include: 1) the recognition of immunologically relevant antigen drainage from the CNS; 2) a better knowledge of the

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distribution and functional properties of antigen-presenting cells (APC) residing in different CNS compartments; and 3) the identification of signals regulating leukocyte recruitment to the inflamed CNS.

The CNS lymphatic drainage

Despite the lack of classically defined lymphatics, the CNS contains fluid compartments thought to represent a modified lymphatic system. These include the interstitial fluids surrounding neurones and glia, the perivascular spaces lined by the subendothelial basal lamina, and the CSF filling the ventricles and the subarachnoid space (1). Because the CSF acts as a 'sink' removing products of the cerebral metabolism into the venous blood, it is conceivable that antigens draining into the CSF enter the venous sinuses through the arachnoid villi and reach the spleen through the blood. The fact that a robust immune response is elicited after delivery of particulate antigens (tissue grafts or pathogens) to the subarachnoid space or ventricles, but not to the CNS parenchyma (6), clearly indicates that antigen may readily drain from this compartment to lymphoid organs. Moreover, there is now considerable evidence for significant drainage of antigens from the brain to cervical lymph nodes. This lymphatic pathway involves direct drainage of CSF and interstitial fluid draining along perivascular pathways into nasal lymphatics through the cribriform plate (1, 7). CSF from the spinal subarachnoid space has been shown to drain to lumbar and, possibly, thoracic lymph nodes. An important role for cervical lymphatics in the immune response against CNS-associated antigens is now supported by several studies in experimental models of CNS infection or injection of exogenous soluble antigen into the CNS. Although not yet demonstrated, transport of myelin self-antigen from the inflamed CNS to CNS-associated lymph nodes could be of key importance in the development and/or maintenance of autoimmune responses in the human demyelinating disease multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis (EAE).

Antigen-presenting cells in the CNS

APC, like dendritic cells and macrophages, are present in virtually all organs and are thought to play a key role in the control of tissue-specific inflammation. Dendritic cells are the most potent APC with the unique ability to take up antigens in peripheral tissues and to migrate to lymphoid organs to initiate immune responses (8). There is growing evidence that dendritic cells also maintain and regulate peripheral T-cell tolerance. Tissue-resident macrophages have been implicated in the local regulation of cellular immune responses through presentation of phagocytosed antigen and activation of tissue-infiltrating T cells. Both the distribution and functional phenotype of these APC populations in the CNS are thought to reflect an adaptation to the unique structure and micro-environment of the CNS.

Key messages

- Immune responses in the central nervous system are strongly influenced by the presence of brain barriers, the immunosuppressive neural environment, and the unique phenotype and distribution of CNS resident immune cells.
- The delicate balance between immune privilege and immune surveillance in the central nervous system is achieved through a complex and highly regulated network of interactions between brain cells and the immune system.

Dendritic cells

It has long been recognized that dendritic cells are excluded from the CNS parenchyma proper. This makes it possible that certain neurotropic viruses (eg, rabies, herpes virus simplex type-1, polio virus), which are able to enter peripheral nerve endings and to spread through the CNS by means of intra-axonal transport, are not immediately recognized by the immune system. More recently, however, it has been recognized that rich networks of dendritic cells are present in the meningeal layers (dura, arachnoid and pia mater), in the stroma and, possibly, in the epithelial layer of the choroid plexuses (9). Thus, CNS-associated dendritic cells appear to be in key positions to contact potential pathogens or self-antigens entering or leaving the CNS through the blood–CSF barriers and to transport them to the cervical lymph nodes or, via blood circulation, to the spleen. In the normal CNS, dendritic cells express major histocompatibility (MHC) class II, but not costimulatory molecules, resembling immature dendritic cells of peripheral tissues, specialized for antigen capture. The finding that choroid plexus dendritic cells express the anti-inflammatory cytokine interleukin-10 (IL-10) suggests that these cells may have an immunosuppressive role and contribute to maintain immune tolerance to CNS antigens (10). Interestingly, infection of choroid plexus dendritic cells by HIV-1 has been described in patients with AIDS. This finding indicates that choroid plexus dendritic cells may represent a potential reservoir of HIV-1 in the CNS

and initiate virus-specific immune responses (11). Recent studies have shown that dendritic cells accumulate in CNS perivascular spaces and even infiltrate the CNS parenchyma under inflammatory conditions (eg, during bacterial and parasitic infection and in EAE) (12, 13), suggesting that dendritic cells recruited from the blood circulation could play an important role in the local regulation of CNS-directed T-cell responses. This is an issue that needs to be further investigated and may provide new insights into the pathogenic mechanisms underlying neuroinflammatory diseases.

CNS-associated macrophages

CNS-associated macrophages comprise cells that exhibit scavenging and phagocytic functions and reside in the meninges, choroid plexuses and perivascular spaces of CNS blood vessels, outside the neural parenchyma proper (14). Perivascular macrophages, as a component of the blood–brain barrier (BBB), are viewed as the first line of defence against blood-derived pathogens and the first immunoregulatory elements interacting with leukocytes extravasating into the CNS tissue (15). Owing to their constitutive expression of MHC class I and class II molecules and based on studies with bone-marrow chimeras and *ex vivo* functional studies, CNS-associated macrophages are thought to play a key role as APC for the restimulation of encephalitogenic T cells in CNS autoimmune diseases (14, 15).

Microglia

Despite sharing a common monocytic progenitor with macrophages present in other tissues or associated with the CNS, microglia display a unique, perhaps less differentiated, phenotype, which is thought to be under the strict control of the neural microenvironment (3, 4). Compared with CNS-associated macrophages, microglia exhibit a ramified morphology, a very slow turnover rate, no phagocytic or endocytic activity, and much lower levels of CD45, MHC class II molecules, complement receptor 3 and Fc receptors. Upon CNS injury and depending on the extent of neuronal damage, microglia progressively lose their ramified morphology and acquire properties of full-blown macrophages (16). Because microglia up-regulate expression of MHC class II molecules in essentially all known CNS pathologies, MHC class II-restricted presentation of processed antigenic peptides to $CD4^+$ T helper cells (Th) is thought to be a key function of activated microglia (16, 17). Under inflammatory conditions, microglia also express a number of adhesion and costimulatory molecules (lymphocyte function-associated antigen 1 (LFA-1), CD40, CD54, CD80/CD86) that are involved in optimal T-cell activation. Similar to what has been observed with macrophages, signals from Th1 cells, both membrane bound signals (eg, the CD40-CD154 pathway) and secreted cytokines (particularly interferon-γ), are essential for promoting microglia APC function (17).

In vitro studies with long-term cultured or acutely isolated microglia from the rodent or human CNS have shown that microglia, once activated, are good stimulators of T-cell effector functions. Although comparable to dendritic cells in the restimulation of primed $CD4^+$ T cells, microglia are much less efficient than dendritic cells in the priming of naïve T cells (18). Similarly to macrophages, microglia produce the Th1-inducing cytokines IL-12 and IL-18, and restimulate Th1 cells with high efficiency, suggesting that they may contribute to the intracerebral skewing of Th1 responses during CNS infection and autoimmunity.

Microglia are also efficient in inducing Th2 responses and might contribute to the establishment of an anti-inflammatory milieu (17, 19). The observations that antigen-presenting microglia can induce T-cell apoptosis (20) and that microglia express Fas-ligand in the inflamed CNS (21) suggest a regulatory role for microglia in limiting spreading of immune responses. Anti-inflammatory mediators (IL-10, TGF-β and prostaglandin E_2) secreted by activated microglia are also likely to contribute to the down-regulation of intracerebral immune reactivity (17, 22). The above studies highlight the fact that, similarly to other APC, microglia are highly versatile cells capable of amplifying, down-regulating or shaping the pro- and anti-inflammatory nature of cellular immunity depending on the environmental context.

Astrocytes

Astrocytes are the most abundant CNS glial cell population, with a primary role in the maintenance of CNS homeostasis, including control of neuronal survival/activity, regulation of BBB function, and reparative processes. Despite the existence of studies suggesting a role for astrocytes as CNS APC, these glial cells are thought to have only a marginal role in stimulating antigen-specific T-cell responses (14, 17). This is supported by observations that reactive astrocytes express MHC class II molecules very rarely and, possibly, only under strong inflammatory conditions (16). Astrocytes also fail to express costimulatory molecules important for T-cell activation and to produce key proinflammatory (eg, IL-1, tumour necrosis factor-α) and immunoregulatory (eg, IL-12, IL-18) cytokines (17). *In vitro* studies in which the APC function of astrocytes has been directly compared with that of microglia and dendritic cells have demonstrated that astrocytes fail to process native proteins and to prime T cells, and that they

restimulate T cells (predominantly Th2 cells) only in the presence of antigenic peptides (17, 18). As a result of their ability to produce mediators with anti-inflammatory activity (prostaglandin E_2, TGF-β), to suppress T-cell activation induced by other APC, and to inhibit production of proinflammatory cytokines (eg, IL-12) and MHC class II expression in microglia/macrophages, astrocytes have been proposed to have an important function in limiting immune-mediated inflammation (17).

Leukocyte traffic to the CNS

There are three potential routes of entry for circulating leukocytes into the CNS: across the BBB into the CNS parenchyma; across the epithelia of the choroid plexuses into the CSF of the ventricular system; and across the arachnoid membrane into the subarachnoid space and, from here, through the pia and basal lamina to the CNS parenchyma.

Although the BBB is thought to have an active role in limiting the passage of immune molecules and cells from the blood into the neural parenchyma, its ability to act as a physical barrier for immune cells is not absolute. Activated T cells, independently of their antigenic specificity, can cross the intact BBB, and cells of the monocyte/macrophage lineage are constantly recruited from the blood circulation to replenish the pool of CNS perivascular macrophages and intraparenchymal microglia (23).

Under normal conditions, very few T cells enter the CNS; however, when the BBB is breached (as occurs in infectious and autoimmune CNS disorders), large numbers of T cells and other blood-born cells can have access. Thus far, the events that control entry of T cells into the CNS have been mainly investigated in the animal model of EAE and have provided novel insights into the pathogenesis and treatment strategies of autoimmune CNS diseases. Under inflammatory conditions, activated leukocytes penetrate the BBB according to the multi-step model of leukocyte–endothelial cell recognition (24). This involves sequential interactions of adhesion molecules with their counter-receptors on activated endothelial cells and leukocytes, as well as of chemokines with their receptors. The adhesion molecules CD54 (intercellular adhesion molecule-1, ICAM-1) and CD106 (vascular cell adhesion molecule-1, VCAM-1) are up-regulated on the endothelial cells of inflamed cerebral vessels and mediate leukocyte adhesion via their respective ligands LFA-1 and the α4-integrins, α4β1 and α4β7 (25). *In vivo* antibody inhibition studies have implicated α4β1-integrin/VCAM-1 (vascular cell adhesion molecule-1) interactions in homing of encephalitogenic T cells to the CNS in EAE and virus-induced encephalitis (25). B cells and dendritic cells have also been shown to accumulate in the CNS during infectious and autoimmune diseases, but the signals required for their extravasation across the BBB remain poorly defined (13, 23).

During the past decade, chemokines have emerged as key molecules regulating leukocyte homing to the inflamed CNS (26). Chemokines are small secreted proteins which, based on the relative position of the first two cystein residues, have been classified into four groups (CXC, CC, C and CX3C) (27). All the chemokine receptors identified thus far are seven-transmembrane-spanning, G-protein-coupled receptors. Most chemokines act on distinct leukocyte populations, and each leukocyte population has receptors for and responds to many chemokines, indicating a complex and partially redundant network. Extensive studies carried out in the CNS of patients with multiple sclerosis or in the animal model, EAE, indicate that increased intracerebral expression of the CXC chemokine IP-10 and the CC chemokines RANTES, macrophage inflammatory protein (MIP)-1α, MIP-1β, and monocyte chemoattractant protein (MCP)-1 correlates with the presence of CNS inflammatory lesions (28). Among CNS resident cells, activated cerebrovascular endothelial cells, astrocytes and microglia have all been identified as major sources of chemokines under inflammatory conditions (29). Studies in mice lacking specific chemokines or chemokine receptors point to a major role of MCP-1 and CCR2 and, to a lesser extent, of MIP-1α and CCR1 in the pathogenesis of EAE, thus suggesting that these molecules may represent potential therapeutic targets in the treatment of multiple sclerosis (30).

Although poorly investigated, the possibility exists that choroid plexus epithelia constituting the blood–CSF barrier might play a key role in the communication between the CNS and the immune system. Increased expression of adhesion molecules, such as ICAM-1, VCAM-1 and mucosal addressin cell adhesion molecule-1 (MAdCAM-1), in choroid plexus epithelia during EAE suggests that these molecules could facilitate the entry of immune cells into the CSF, a hallmark of infectious and autoimmune CNS diseases, as well as the exit of intraventricular leukocytes (31). Choroid plexus epithelia could also contribute to the synthesis of cytokines and chemokines the levels of which are increased in the CSF during CNS inflammation.

Conclusion

Under physiological conditions, a continuous and tightly regulated communication between the CNS and the immune system is necessary to maintain the delicate balance between immune privilege and immune surveillance. While CNS integrity and func-

tion probably benefit from maintenance of an immune privileged status, this may occur at the expenses of efficient recognition and elimination of pathogenic micro-organisms, leading to the establishment of persistent infections and chronic immunopathological states. From the above discussed, it is, however, apparent that distinct CNS compartments exist in which immune reactivity is regulated differently. Like most peripheral tissues, the compartment comprising the meninges, subarachnoid/ventricular spaces and choroid plexuses contains professional phagocytes and APC, is readily accessible to blood-derived infectious agents and immune cells or signals, and connects to secondary lymphoid organs. All these represent preferential sites for the development of inflammation in immune-mediated neurological diseases. Although representing an unfavourable environment for the activation of both innate and acquired immune responses, under certain pathological conditions the CNS tissue itself can be involved in shaping intracerebral immune reactivity. Current evidence indicates that perivascular macrophages residing immediately behind the BBB endothelium and activated intraparenchymal microglia may participate in the stimulation and regulation of immune responses. Given the involvement of both the T-cell and B-cell arms of the immune system or either one of them in a number of neurological diseases associated with neurone dysfunction (eg, paraneoplastic neurological disorders, Rasmussen's encephalitis) or myelin loss (eg, multiple sclerosis), a better knowledge of how different CNS compartments and cells residing therein interact with the immune system may help to shed light into the mechanisms underlying the loss of immunological tolerance and triggering of pathogenic immune responses towards CNS antigens.

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References

1. **Cserr HF, Knopf, PM.** Cervical lymphatics, the blood-brain barrier and immunoreactivity of the brain. In: Keane RW, Hickey WF, eds. *Immunology of the nervous system.* New York, NY: Oxford University Press; 1997: 134–54.
2. **Bechmann I, Mor G, Nilsen J, Eliza M, Nitsch R, Naftolin F.** FasL (CD59L, Apo1L) is expressed in the normal rat and human brain: evidence for the existence of an immunological barrier. *Glia* 1999; 27: 62–74.
3. **Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, et al.** Down-regulation of the macrophage lineage through interaction with OX2 (CD200). *Science* 2000; 290: 1768–71.
4. **Neumann H, Wekerle H.** Neuronal control of the immune response in the central nervous system: linking brain immunity to neurodegeneration. *J Neuropathol Exp Neurol* 1998; 57: 1–9.
5. **Wenkel H, Streilein JW, Young MJ.** Systemic immune deviation in the brain does not depend on the integrity of the blood-brain barrier. *J Immunol* 2000; 164: 5125–31.
6. **Perry VH.** A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation. *J Neuroimmunol* 1998; 90: 113–21.
7. **Weller RO, Engelhardt B, Phillips MJ.** Lymphocyte targeting of the central nervous system: a review of afferent and efferent CNS-immune pathways. *Brain Pathol* 1996; 6: 275–88.
8. **Banchereau J, Steinman RM.** Dendritic cells and the control of immunity. *Nature* 1998; 392: 245–52.
9. **McMenamin PG.** Distribution and phenotype of dendritic cells and resident tissue macrophages in the dura mater, leptomeninges, and choroid plexus of the rat brain as demonstrated in wholemount preparations. *J Comp Neurol* 1999; 405: 553–62.
10. **Serot J-M, Béné M-C, Foliguet B, Faure CG.** Monocyte-derived IL-10 dendritic cells in choroid plexus epithelium. *J Neuroimmunol* 2000; 105: 115–9.
11. **Hanly A, Petito CK.** (1998) HLA-DR positive dendritic cells of the human choroid plexus: a potential reservoir of HIV in the central nervous system. *Hum Pathol* 1998; 29: 88–93.
12. **Fisher H-G, Bonifas U, Reichmann G.** Phenotype and functions of brain dendritic cells emerging during chronic infection of mice with *Toxoplasma gondii*. *J Immunol* 2000; 164: 4826–34.
13. **Serafini B, Columba-Cabezas S, Di Rosa F, Aloisi F.** Intracerebral recruitment and maturation of dendritic cells in the onset and progression of experimental autoimmune encephalomyelitis. *Am J Pathol* 2000; 157: 1991–2002.
14. **Sedgwick JD, HickeyWF.** Antigen presentation in the nervous system. In: Keane RW, Hickey WF, eds. *Immunology of the nervous system.* New York, NY: Oxford University Press; 1997: 364–418.
15. **Thomas WE.** Brain macrophages: on the role of pericytes and perivascular cells. *Brain Res Rev* 1999; 31: 42–57.
16. **Kreutzberg GW.** Microglia: a sensor for pathological events in the CNS. *Trends Neurosci* 1996; 19: 312–8.
17. **Aloisi F, Ria F, Adorini L.** Regulation of T cell responses by central nervous system antigen presenting cells: different roles for microglia and astrocytes. *Immunol Today* 2000; 21: 141–7.
18. **Aloisi F, Ria F, Columba-Cabezas S, Hesse H, Penna G, Adorini L.** Relative efficiency of microglia, astrocytes, dendritic cells and B cells in naïve $CD4^+$ T cell priming and Th1/Th2 restimulation. *Eur J Immunol* 1999; 29: 2705–14.
19. **Ma N, Streilein JW.** T cell immunity induced by allogeneic microglia in relation to neuronal retina transplantation. *J Immunol* 1999; 162: 4482–9.
20. **Ford AL, Foulcher E, Lemckert FA, Sedgwick JD.** Microglia induce CD4 T lymphocyte final effector function and death. *J Exp Med* 1996; 184: 1737–45.
21. **Kohji T, Matsumoto Y.** Coexpression of Fas/FasL and Bax on brain and infiltrating T cells in the central nervous

system is closely associated with apoptotic cell death during autoimmune encephalomyelitis. *J Neuroimmunol* 2000; 106: 165–71.
22. **Aloisi F.** Immune function of microglia. *Glia* 2001 (in press).
23. **Hickey WF.** Leukocyte traffic in the central nervous system: the participants and their roles. *Semin Immunol* 1999; 11: 125–37.
24. **Springer TA.** Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 1994; 76: 301–14.
25. **Engelhardt B.** The blood-brain barrier. In: Russel WC, ed. *Molecular biology of multiple sclerosis*. New York, NY: John Wiley & Sons Ltd; 1997: 137–60.
26. **Baggiolini M.** Chemokines and leukocyte traffic. *Nature* 1998; 392: 565–8.
27. **Zlotnik A, Yoshie O.** Chemokines: a new classification system and their role in immunity. *Immunity* 2000; 12: 121–7.
28. **Glabinski AR, Ransohoff RM.** Chemokines and chemokine receptors in CNS pathology. *J Neurovirol* 1999; 5: 3–12.
29. **Hesselgesser J, Horuk R.** Chemokine and chemokine receptor expression in the central nervous system. *J Neurovirol* 1999; 5: 13–26.
30. **Gerard C, Rollins BJ.** Chemokines and disease. *Nat Immunol* 2001; 2: 108–15.
31. **Engelhardt B, Wolburg-Buchholz K, Wolburg H.** Involvement of the choroid plexus in central nervous system inflammation. *Microsci Res Tech* 2001; 52: 112–29.