INFLAMMATION AND AUTOIMMUNITY: A NERVOUS SYSTEM PERSPECTIVE

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Introduction

Higher mammals have an adaptive immune system able to respond adequately and rapidly and to eliminate any foreign pathogens, microorganisms, and molecules that may otherwise represent a threat for health and integrity. Having this defence machinery, however, comes with a price: the risk that the host will develop an autoimmune response towards one of its own self antigens.

For more than a hundred years, the brain has been widely considered to be an immune-privileged site because of its protection by a subtle yet powerful blood–brain barrier (BBB), a lack of lymphocyte drainage, and the absence of in situ antigen-presenting cells (APCs). Over the last decade, recent observations have amended this concept, and the notion of immune surveillance, by which the brain is continuously surveyed by immune cells, now prevails. However, the presence of immune cells within the central nervous system (CNS), behind the BBB, brings the risk of inflammation, and exposes the brain to a potential autoimmune threat. Despite early evidence concentrating on the pathogenic role of autoimmune T cells, recent evidence suggests that B-cell and antibody involvement is important during the effector stages of autoimmunity. Deciphering the role of the cellular and humoral immune components within the CNS should enable us to understand autoimmune pathophysiological mechanisms and exploit this knowledge for immune-based therapeutic purposes.

This chapter reviews the recent literature on several concepts in neuroimmunology and the role of the immune system in the healthy brain, as well as driving the disease process, with a particular emphasis on autoimmune responses leading to neurological diseases.

Immune privilege versus immune surveillance

The concept of immune privilege was originally cited in literature related to transplantation. The allograft of genetically mismatched tissues onto the brain displayed prolonged survival compared with other organs such as skin (Head and Griffin 1985, Simpson 2006), highlighting the fact that immune responses within the brain were hard to elicit (Carson et al 2006). It is partly due to the existence of the so-called BBB that many substances are kept out of the brain parenchyma. First introduced by Paul Ehrlich in 1885, the idea that the brain was located behind a barrier protecting it from harmful substances in the bloodstream has been largely accepted and verified. Indeed, dyes injected into the blood supply stain the tissues of most
organs except the brain, and virus clearance from the brain parenchyma has been shown to be slow and delayed (Stevenson et al 2002). The existence of the BBB also provides protection against systemic immune activation, which could be detrimental to the brain. The brain is particularly susceptible to an increase in tissue volume due to space limitations imposed by the dura mater and the skull, and, once damaged, neurons display a poor capacity to be replaced. The BBB is a specialized system composed of endothelial cells that are joined by tight occluding junctions, and the abluminal side of these endothelial cells is surrounded by basement membranes and circled by the end-feet of astrocyte processes (Fig. 1.1). The BBB therefore allows only blood gases, small molecules, and fluids to cross, apart from some active transport processes. Thus, as a result of restricted permeability and the expression of low levels of adhesion molecules, the BBB is a limiting factor not only for the delivery of therapeutic agents, but also for the migration of immune cells into the CNS (Hickey 2001).

However, the BBB is not absolute, even in the healthy brain, and it is now understood that immune cells survey the CNS under homeostatic conditions, a phenomenon that can be defined as immune surveillance. The notion of immune surveillance involves the presence of immune cells within the brain and implies low-level trafficking through the organ. Indeed, T cells, B cells, and APCs are found in the CNS of healthy animals, but at very low numbers (Hickey and Kimura 1988, Ransohoff et al 2003, Engelhardt and Ransohoff 2005). The trafficking of these cells is reportedly slow. Most of the activated lymphocytes remain

**Fig. 1.1** The blood–brain barrier (BBB). Formed by endothelial cells, astrocytes, and pericytes, the BBB is a specialized system controlling access to the central nervous system. Tight occluding junctions between endothelial cells as well as a basement membrane and astrocyte end-feet moderate trans- and paracellular traffic.
in the non-parenchymal sites of the CNS (perivascular, meningeal, and P-selectin-expressing subarachnoid spaces), leaving the CNS parenchyma largely untouched (Carson et al 2006). This may explain why brain grafts survive better in brain parenchyma than in skin (Head and Griffin 1985) and why robust proinflammatory T-cell responses to grafted tissues and pathogens are readily triggered within the zones where T cells can be detected (i.e. ventricles, the meninges, and the subarachnoid spaces) (Perry 1998). All of these structures are filled, or in contact, with the cerebrospinal fluid (CSF), which is used as a route of migration by which cells traffic through the brain under physiological conditions. Although the exact entrance point remains largely unknown (it is assumed to be the subarachnoid space), the lymphocyte turnover between blood and CSF happens at least twice a day, providing a continuous flux through the CNS of lymphocytes with different specificities. In humans, healthy CSF contains <5 leucocytes per mm³. Those cells are essentially T cells (80%), mainly from the CD4+ subset, and display a central memory phenotype (CD4+, CD45RO+, CD27+, CXCR3+) (Engelhardt and Ransohoff 2005). Thus, although the status of an ‘immune-privileged organ’ still holds true, it is important to understand that this definition has been revisited, and now refers to a slow adaptive immune response within the brain parenchyma. From ‘immune privileged’ the brain has become ‘immune specialized’.

**Immune surveillance of the brain**

During the immune response priming phase, physical contacts with an APC provide activation signals to naive T cells. The priming phase generally happens in lymphoid organ structures where APCs and lymphocytes are in a micro-environment favouring close interactions (such as the cervical lymph nodes). After this contact, activated cells home to sites of immune activation and take part in the mounting of the immune response. Thus, the ability of the brain to develop an immune response depends on several features: an encounter between immune cells, including professional APCs, with CNS antigens; an interaction between the T cells and APCs (as T cells are unable to be activated by unprocessed native antigens); and some sort of lymphatic drainage to enable afferent and efferent arms of an immune response to interact.

**Interactions with Antigen-presenting Cells**

APCs of haematopoietic origin from the monocyte lineage perform specialized functions and are involved in immune surveillance. This heterogeneous group is divided into three main types. First, microglial cells migrate before birth, localize within the parenchyma, and constitute the main cell type responsible for the primary defence against pathogens entering the brain. They survive for extended periods of time. The second APC type is the perivascular macrophage, which, in contrast, is continuously being replaced in the CNS from healthy bone marrow (Hickey and Kimura 1988). These macrophages are localized in perivascular spaces surrounding small and medium-sized cerebral vessels, also called Virchow–Robin spaces (Williams et al 2001). Thus, under resting conditions these macrophages are continuously entering the CNS across a normal BBB, again illustrating the existence of exchange between the CNS and the immune system (Hickey 1999, Bechmann et al 2001). The third type of APCs are macrophages and dendritic cells that gather within the meninges and choroid plexus (the site of CSF production). It has been proposed that central memory T cells carry out routine
immune surveillance of the CNS by searching within the CSF-filled subarachnoid spaces for recall antigens presented by these APCs (Engelhardt and Ransohoff 2005).

**Encounter with Antigens and Lymphatic Drainage**

In the healthy brain, soluble antigens can drain via the CSF along the perivascular and subarachnoid spaces through the paper-thin cribriform plate into the lymphatics of the nasal submucosa and into the cervical lymph nodes (Cserr and Knopf 1992, Cserr et al 1992). This is important because the lack of classic lymphatics in CNS tissue was first thought to prevent the delivery of antigens to lymphoid organs, where the high density of immune cells could trigger an efficient response. Thus, the CSF might partially act as lymph for the CNS (Weller et al 1996). Although most of these studies have been performed on rodents and ruminants, anatomical studies have highlighted similar structures in humans, and the pathway between the CNS and cervical lymph nodes is thought to be the same. Moreover, as previously stated, the presence of APCs within the CNS along the perivascular and the subarachnoid spaces argues that antigen-driven immune responses can be stimulated within these spaces. However, the spinal cord is a specific case. It appears that migration of lymphocytes across non-inflamed spinal cord parenchyma/vessels takes place and is of a different nature, involving α4 integrins (Vajkoczy et al 2001, Ransohoff et al 2003, Engelhardt and Ransohoff 2005).

In summary, it seems probable that even though the brain displays slower immune reactions, all components of an efficient immune response can be found in the CNS and would be able to interact with each other in order to take part in inflammation and/or autoimmune responses.

**The immune and inflammatory responses in the brain**

Throughout the body, inflammation has two general purposes: tissue homeostasis and tissue defence against pathogens. As described earlier, the healthy brain is constantly undergoing immune surveillance. Low numbers of T lymphocytes are detected within the CNS, but they are mainly confined to the perivascular and meningeal spaces. Inflammation in the brain starts a chain of events including infiltration of lymphocytes, secretion of cytokines by mononuclear cells, and modification of adhesion molecule expression on BBB endothelium and choroid plexus epithelium (Engelhardt and Ransohoff 2005). Two major possibilities should be considered: first, T cells could face antigens outside the CNS and then cross the BBB or, alternatively, the encounter could happen within the brain after naive (non-activated) T cells have crossed the BBB. Both of these mechanisms are likely to occur in different human disease states.

**Proposed Mechanism 1: Lymphocytes Require Activation Before Crossing the Blood–Brain Barrier**

This mechanism implies that an immune response will be promoted outside the brain and that T cells would be activated before trafficking to the CNS through the BBB. According to our current understanding, the essential requirement of lymphocyte entry into the CNS is activation. The activation status is the most important hallmark, regardless of the antigen specificity. However, after a few hours neuroantigen specificity is needed for lymphoblast persistence in
the CNS (Hickey et al 1991, Hickey 2001). Activated T cells can detect antigens within the CNS as readily as antigens in other organ sites.

In addition, it is widely accepted that, under certain pathophysiological conditions, the access to the brain parenchyma is increased via breakdown of the BBB. In a general fashion, BBB breakdown, or alterations in transport systems, play an important role in the pathogenesis of many CNS diseases, such as HIV-1 encephalitis, Alzheimer disease, ischaemia, tumours, Parkinson disease, and CNS autoimmune diseases. Proinflammatory substances and specific disease-associated proteins often mediate such BBB dysfunction. Although the breakage of the BBB is not necessary or sufficient to cause autoimmunity, it certainly potentiates the risk of triggering an autoimmune response if the individual humans carry circulating lymphocytes that are specific for CNS antigens. Additionally, the fact that peripherally activated T/B lymphocytes can enter and attack CNS tissue suggests the possibility that ‘molecular mimicry’ occurs in neurological diseases (defined as epitope cross-reactivity between an infectious agent or tumour antigen and a self antigen). Molecular mimicry has been postulated in multiple sclerosis, stiff person syndrome, and paraneoplastic disorders (Wekerle and Hohlfeld 2003, Hassin-Baer et al 2004, Roberts and Darnell 2004) (Table 1.1), but the best example is Guillain–Barré syndrome, an autoimmune disease of the peripheral nervous system that is triggered in 25% of cases by intestinal infection with Campylobacter jejuni (Ogawara et al 2000, Dalakas 2006).

**PROPOSED MECHANISM 2: LYMPHOCYTES DO NOT REQUIRE ACTIVATION BEFORE CROSSING THE BLOOD–BRAIN BARRIER**

Alternatively, recent results suggest that non-activated T cells can enter the CNS parenchyma in an animal model of CNS inflammation, and can be activated in situ by cognate antigen (McMahon et al 2005).

**Autoimmune cellular responses within the brain**

Most of the understanding of CNS inflammation and autoimmunity is derived from the study of multiple sclerosis.

**T CELLS**

Until recently, most of the research on neurological autoimmune diseases has been deciphering the role of T lymphocytes in multiple sclerosis. This focus on T lymphocytes is because inflammatory lesions in multiple sclerosis are infiltrated predominantly by CD4+ T cells (but also macrophages and some B cells). The study of inflammation in multiple sclerosis has taken advantage of a murine animal model called autoimmune experimental encephalomyelitis (EAE), which displays symptoms of demyelination after immunization. The focus on T cells also probably relates to the fact that EAE (and, to some extent, autoimmune experimental neuritis) can be induced in naive recipients by adoptive transfer of myelin-specific T cells (Ransohoff et al 2003, Dalakas 2006). The detailed mechanisms by which T cells enter inflamed tissues of the CNS remains to be clarified. Activation of T cells facilitates their adhesion and trafficking through the BBB, and numerous studies have reported the changes in adhesion molecule expression on migrating lymphocytes (Ransohoff et al 2003, Mrass and Weninger
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a Detection of DNA and nuclear antigens has been associated with lupus activity in these patients.
b Non-exhaustive list.
– Undetermined or unknown.
2006). Notably, the involvement of α₄β₁ integrins and their ligand, V-CAM-1, has been considered important in trafficking (Baron et al 1993, Brocke et al 1999). Further support for the role of adhesion molecules is the successful use of natalizumab in multiple sclerosis. Indeed, despite a number of concerns about its safety, by blocking V-CAM this human monoclonal antibody directed towards α₄β₁ and α₄β₇ integrins reduces the occurrence of clinical relapses and decreases the formation of magnetic resonance imaging (MRI) multiple sclerosis lesions compared with placebos (Polman et al 2006, Yousry et al 2006).

The immunophenotype of the disease-mediating cells has also been subject to considerable scrutiny. In multiple sclerosis, CNS-infiltrating T cells were thought to be primarily interferon gamma (IFN-γ)-producing CD4⁺ T cells (Th1 phenotype). This impression has evolved and several reports have highlighted, mainly in EAE, the role of the newly recognized IL-17-secreting CD4⁺ subset that originates from a different lineage to Th1 (IFN-γ) and Th2 (responsible for the production of IL-4 and IL-10) cells (Iwakura and Ishigame 2006, McKenzie et al 2006, McFarland and Martin 2007). On the other hand, the presence of CD8⁺ (cytotoxic) cells has also been reported in human multiple sclerosis lesions (Traugott et al 1983). Some authors have recently suggested that multiple sclerosis lesions are initiated by CD4⁺ T cells, but that the amplification and damage are mediated by CD8⁺ T cells (McFarland and Martin 2007).

**B Cells**

Ten years ago, pioneering studies on rats provided evidence that activated B cells are able to cross an intact BBB in search of their antigens and to differentiate into immunoglobulin (IgG)-producing plasma cells within the CNS (Knopf et al 1998). Additionally, data from EAE and multiple sclerosis lesions indicate that B cells are able to traffic through the BBB and play a role in the initiation and development of disease within the CNS (Genain et al 1999, Raine et al 1999). Thus, B cells are found in multiple sclerosis lesions and play an active role in inflammation. Effector mechanisms include antibody secretion (see below), activation-dependent release of cytokines, complement binding, and the reciprocal activation of T cells via antigen presentation (Archelos and Hartung 2000). Interestingly, rituximab, a monoclonal antibody targeting the B-cell-specific CD20, has produced the unexpected finding of a rapid reduction in acute multiple sclerosis activity assessed by MRI (Edwards et al 2004, McFarland and Martin 2007). However, rituximab may act by decreasing the B-cell-mediated presentation of antigens to T cells, and thus reducing the T-cell immune response, rather than by only preventing antibody production.

As part of the chain of events after cytokine secretion by activated B cells, non-specific tissue damage can also be mediated by activated macrophages and microglial cells via proteolytic enzymes, cytotoxic cytokines, and cell death-inducing surface molecules such as Fas-ligand (Bauer et al 2001).

**Autoimmune antibody responses within the brain**

Antibodies, produced by plasma cells after clonal expansion of antigen-specific B cells, can be readily detected in several neurological autoimmune diseases. Typical examples are ‘oligoclonal bands’, strong and narrow IgG bands observed after protein electrophoresis of
the CSF of patients suffering from multiple sclerosis, subacute sclerosing panencephalitis, neurosyphilis, and other infectious or inflammatory brain disorders (Ransohoff et al. 2003). However, defining the pathogenic role of autoantibodies has been a major difficulty and an ongoing theme of research in many autoimmune diseases. Indeed, the distinction between antibodies as ‘biomarkers’ and antibodies as ‘mediators’ of autoimmunity remains difficult. Four main criteria attest to the pathogenicity of autoantibodies:

1. detection of measurable autoantibodies;
2. antibody presence in target tissue;
3. induction of disease after passive transfer of antibodies in an animal model;
4. clinical improvement after antibody removal with plasma exchange or intravenous immunoglobulins (Lang et al. 2003).

This pathogenicity has been definitively demonstrated in only a few B-cell-mediated autoimmune diseases, such as myasthenia gravis and Lambert–Eaton myasthenic syndrome (Drachman 1994, Vincent et al. 2000, Dalakas 2006). Further examples of potential pathogenic autoantibodies are discussed in this book (including antibodies against folate receptors, different types of glutamate receptors, and voltage-gated potassium channels). Autoantibodies are present in a number of neurological diseases and their autoantigen targets include a broad spectrum of neuronal-specific and non-neuronal proteins (Table 1.1). Although not proven to be pathogenic in the majority, immunoglobulin-mediated tissue injury in autoimmune diseases is still an attractive hypothesis, given the specificity of antibodies towards their antigen. Mechanisms of antibody-mediated dysfunction may include:

- **Antigen-dependent cellular cytotoxicity**: binding of an antibody to antigen exposing its Fc fragment to Fc receptors expressed on effector cells, namely monocytes and natural killer cells. This results in the lysis of the antigen-expressing cell (Antel and Bar-Or 2006). Some distinct pathological phenotypes of early multiple sclerosis lesions have been observed to be the results of antigen-dependent cellular cytotoxicity (Lucchinetti et al. 2004, Antel and Bar-Or 2006).

- **Antibody-dependent complement-mediated toxicity**: activation of complement by immune complexes. Autoantibody-induced activated complement fragments, such as C3a, C3b, and C5a, form complexes and behave as chemoattractants for lymphocytes and macrophages (Archelos and Hartung 2000). In the case of multiple sclerosis, it has been shown that complement can be activated by antibodies against myelin (Reindl et al. 1999). Additionally, high levels of the lytic membrane complex C5b–9 and immunoglobulins were also detected in multiple sclerosis lesions (pattern II) and are thought to open pores in myelin, causing demyelination (Lucchinetti et al. 2000).

- **Direct effect on function of the cell surface antigens or on the turnover or expression of the channels or receptors**: see examples of myasthenia gravis and Lambert–Eaton myasthenic syndrome.
Identification of the self antigens in neurological autoimmune diseases has been a focus of research for many years. The best example of unquestionable pathogenic autoantibodies in neurological disease remains the finding of autoantibodies against acetylcholine receptors in myasthenia gravis and voltage-gated calcium channels in Lambert–Eaton syndrome.

Relevant findings in other diseases may have been impaired by the phenomenon of epitope spreading. Epitope spreading is defined as the development of an immune response to epitopes distinct from, and not cross-reactive with, the disease-causing epitope, and can be extended to include other proteins within the target tissue. Observed in EAE and in multiple sclerosis, epitope spreading highlights the importance of investigating immune responses as early as possible after the biological onset of disease, at a stage when secondary tissue injury and environmental exposures are limited.

Conclusions
Results from human and animal studies have improved our understanding of the immune response in the brain under resting and disease conditions. At present, we consider that the brain is under constant immune surveillance by an active immune system, although the responses taking place within this organ are reduced because of the anatomical constraints of the BBB. The notion of immune specialization rather than immune privilege seems to reflect more accurately the relationship between the CNS and the immune system. Considerable progress has been made towards elucidating the immunological events surrounding autoimmune diseases within the CNS. These new insights have the potential to provide valuable therapeutic targets, and several clinical trials based on monoclonal antibodies have already shown promising results. More effort should be placed into understanding the very early steps of autoimmunity within the CNS. In that sense, steps towards studying autoimmune diseases during early childhood would appear to be critical.

REFERENCES


