Section 1
Chapter 1

Interactions between the immune and nervous systems

Effectors and determinants of the innate and adaptive immune responses

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Introduction

Multicellular organisms must protect themselves against a plethora of exogenous enemies surrounding them. In the case of microbes, the body relies on several, scaled strategies. Firstly, there are external membranes, such as the skin and mucous membranes, that ward off most potential intruders. The physical barriers are highly efficient, but not perfect; at any time, a few potential pathogens can leak through and invade the tissues. These invaders are dealt with by an intricate system of internal defense, the immune system, which identifies the foreign pathogen and mounts a response with the ultimate aim to neutralize and eliminate it.

There are principally two classes of immune responses, one, the innate immune response, is based on response elements preformed within the body, and thus is immediately available upon contact with a microbial target. The other response type, adaptive immunity, develops following the first contact with the pathogen. It produces specific effector cells and humoral antibodies, which are exclusively programmed to exterminate infectious agents, and it provides an immunological memory (Table 1.1).

Importantly, however, innate and adaptive immune reactions are not strictly separated, but tightly interconnected (Medzhitov, 2001). Thus, in the course of early innate immune reactions, the presentation of antigens to cells of the adaptive immune system is enhanced, while, conversely, ongoing adaptive reactions can trigger or enhance innate reactivity.

The general rules of immune response are of proven validity for most of the body's organs and tissues. They also govern protection of the central nervous system (CNS), although in a modified version that takes into account the particular requirements of these very special tissues. This chapter will discuss how innate and adaptive immune responses function in the CNS to maintain health and to modulate different diseases.

Innate immunity

Innate immune reactivity is the oldest version of immunity. It acts throughout phylogeny, in insects as in mammals, even in plants. Like its adaptive counterpart, the innate immune system must be able to distinguish between foreign structures, which are to be discarded, and the various components of the "self", which must be tolerated. Recognition of infectious non-self is mediated by a limited number of germline-encoded *pattern-recognition receptors* (PRRs), that recognize structural motifs typical for microbial agents, and which trigger rapid inflammatory responses (Medzhitov and Janeway, 1997). Some PRRs can also recognize endogenous "danger signals" (Wagner, 2006), such as mitochondrial components leaking out of a destroyed cell. These receptors alert the immune system to cell damage, independent of microbial infection. Activation of innate immune pathways occurs in the brain classically in infectious diseases of the CNS, but also under "sterile" conditions (Wyss-Coray and Mucke, 2002). Brain injury, neurodegeneration, ischemia, autoimmunity and neoplasia can all give rise to innate immune responses within the CNS tissues. The consequences of innate immune activation in non-infectious CNS diseases are ambiguous. Dependent on the context, the reactions can either mediate damage to neurons or, in contrast, protect them from exogenous insult.

Pattern recognition receptors: TLR, NLR, RLR, and others

Throughout evolution, some structures have remained unchanged while others were either lost or profoundly modified. The PRRs make use of structural motifs that are present in primitive organisms (e.g. bacteria), but which are not expressed in vertebrates. To initiate immune responses, PRRs recognize pathogen-associated molecular patterns (PAMPs). The PRRs are present in

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Table 1.1 Innate and adaptive immunity

Property	Innate immune system	Adaptive immune system
Receptors	PRR fixed in genome	Gene segments, rearrangement, diversity
Responding cells	Immune cells, tissue resident cells (microglia, astrocytes, neurons?)	B cells, T cells
Recognized targets	Conserved molecular patterns	Details of molecular structure: proteins, peptides, carbohydrates
Self/Non-self discrimination	Endogenous PRR ligands as danger signals	Autoreactive T cells and B cells are a component of the normal immune repertoire
Memory	Memory of the species	Individual memory

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PRR: Pattern recognition receptor.

three different compartments: soluble in body fluids, immobilized on cell membranes, and internally within the cell, that is within the cytoplasm. The PRRs belong to different families, namely *toll-like receptors* (TLRs), RIG-like receptors (e.g. RIG-I, MDA5), NOD-like receptors (e.g. NOD-1, NALP-3), C-type lectins (e.g. DC-SIGN), scavenger receptors (e.g. CD36), complement factors (C1q, C3), collectins (mannose-binding lectin) and pentraxins (e.g. CRP) (Lee and Kim, 2007).

The TLRs are the best-characterized signal generating receptors among the PRRs. The *Toll* gene was discovered in *Drosophila* as a gene involved in antimicrobial immunity, but subsequent studies also identified related genes in vertebrates (Hoffmann *et al.*, 1999). Currently we know of 13 mammalian TLR paralogues. The TLRs 1, 2, 4, 5, and 6 are located mainly on the cell surface and recognize primarily bacterial components, while TLRs 3, 7, 8, and 9 are located *within* the cell and mainly recognize viral products (Lee and Kim, 2007). The ligands for TLRs 10, 12 and 13 remain unidentified. The TLR family members are not equally distributed across species; for example, TLR10 is expressed in humans but not in mice; TLR8 is not functional in mice; and TLRs 11, 12, and 13 are expressed in mice but not humans (Baccalà *et al.*, 2007). Multiple negative regulatory mechanisms have also evolved to attenuate TLR signaling to maintain immunological balance (Liew *et al.*, 2005).

Two other families of innate receptors, namely NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs), cooperate with TLRs. The NLRs detect bacteria, whereas the RLRs detect viruses. The NLRs and RLRs trigger responses similar to TLRs (Creagh and O'Neill, 2006; Meylan *et al.*, 2006).

Recognition of self by PRR

Importantly, certain TLRs are activated by *endogenous* ligands, components of the body's own tissues. The TLR4, for example, binds numerous ligands, which include fibrinogen, fibronectin, heparin sulfate, hyaluronan, and heat shock proteins (HSPs) (Kielian, 2006). It should be noted, however, that some initial descriptions of endogenous TLR4 ligands could have been obscured by LPS contamination of the "endogenous" antigen preparations (Bausinger *et al.*, 2002). Independent of this, there is now overwhelming evidence that TLRs can also be activated by endogenous ligands. In addition, endogenous RNA and DNA are recognized by the endosomal nucleic acid recognizing TLRs; additionally, evidence for TLR-independent recognition pathways of endogenous nucleic acids has been obtained (Wagner, 2006). Binding of endogenous nucleotides to TLRs (such as TLR7 and 9) seems to be involved in triggering systemic autoimmunity (Marshak-Rothstein, 2006).

There are additional PRRs specific for host endogenous components; for example, scavenger receptors A and CD36 can recognize apoptotic cells and initiate their phagocytosis (Lee and Kim, 2007). In addition, NALP3, a component of a molecular complex called the inflammasome, is critical for recognizing endogenous danger signals such as extracellular ATP and uric acid crystals (Fritz *et al.*, 2006).

Mutations in NALP3 have been found to be responsible for three rare human autoinflammatory disorders: Muckle–Wells syndrome, familial cold autoinflammatory syndrome, and chronic infantile neurological cutaneous and articular syndrome. Interestingly, these

diseases respond well to treatment with IL-1 receptor antagonist, redirecting attention to IL-1 as a critical mediator of inflammation (Agostini *et al*., 2004).

An additional link between PRR engagement and autoimmunity has emerged in a mouse model of spontaneous systemic lupus erythematosus (SLE). In lupus-prone MRL/l mice, functional deficiency of TLR9 enhanced the development of disease, indicating that TLR9 might be involved in the maintenance of tolerance (Ehlers and Ravetch, 2007).

CNS resident cells and innate immune reactivity

Innate immunity is firmly established within the CNS. Several PRRs are constitutively expressed in CNS cells, others require induction. In particular, CD14 and TLR2 have been detected in circumventricular organs, in the choroid plexus and the leptomeninges, all representing CNS areas that lack a complete blood–brain barrier (BBB) and which are particularly exposed to invading pathogens (Rivest, 2003). Inflamed CNS tissues increase production of functional PRRs. Active multiple sclerosis (MS) lesions display high levels of TLR3 and TLR4 in their local microglial cells (Bsibsi *et al*. 2002).

In general, resident myeloid cells, namely the microglia, are the main players in innate immune responses within the CNS (Aloisi, 2001). However, evidence is emerging that, in addition, astrocytes, the most abundant glial cell population of the CNS, substantially contribute to local innate immune response against a variety of insults (Farina *et al*., 2007).

Low basal levels of TLR4 expression have been identified in microglia in vivo (Bsibsi *et al*., 2002). Accordingly, systemic administration of the TLR4 ligand, LPS, leads to rapid up-regulation of TLR2 in microglia and generates an innate inflammatory response that is readily detected and more prominent in BBB-free areas of the CNS but also extends into the brain parenchyma (Farina *et al*., 2007). Basal levels of TLR3 are noted in the healthy brain, detectable on astrocytes in the hippocampus and striatum (Park *et al*., 2006). Indeed, several recent in vitro studies have confirmed TLR3 as the predominant TLR expressed by astrocytes. Two reports analyzed the complete human TLR repertoire in human fetal astrocytes by quantitative polymerase chain reaction and pointed to TLR3 as the only TLR with consistent expression in the resting condition (Farina *et al*., 2005; Jack *et al*., 2005). The TLR3 was also up-regulated following treatment with inflammatory cytokines such as IL-1β, IFN-β and IFN-γ (Farina *et al*., 2005).

The central role of astrocytes in regulating neuro-inflammation was recently demonstrated in vivo (Brambilla *et al*., 2005; van Loo *et al*., 2006a). Transgenic mice were generated in which NF-kB, an important transcription factor controlling innate immune responses, was selectively inactivated in astrocytes (Brambilla *et al*., 2005). While these mice displayed normal spinal cord architecture, their functional recovery after injury was dramatically improved. These observations correlated with a drop in leukocyte recruitment into the lesioned area due to the reduced NF-kB-dependent expression of CXCL10 and CCL2 (Brambilla *et al*., 2005). Similarly, blockade of the NF-kB pathway in neuroectodermal cells of the CNS (including neurons, astrocytes and oligodendrocytes) led to a consistent decrease in pro-inflammatory gene expression during experimental autoimmune encephalomyelitis (EAE) (van Loo *et al*., 2006b). In summary, the NF-kB pathway in astrocytes is a key regulator of inflammation in the CNS and its inhibition has beneficial effects on tissue regeneration.

In addition to glial cells, neurons can also participate in innate immune reactions. While the production of IFN-γ by neurons was observed some time ago (Neumann *et al*., 1997a), recent evidence shows that neurons also produce type I interferons (reviewed in Paul *et al*., 2007). There is also recent evidence that also establishes the expression of TLR3 (Lafon *et al*., 2006) and TLR8 (Ma *et al*., 2006) by neurons.

Innate immune activation in the periphery can promote CNS autoimmunity

Several lines of evidence link infections in peripheral tissues and microbial structures to autoimmune processes in the CNS. In a model of spontaneous EAE in transgenic mice, EAE developed more readily in mice housed in a non-sterile facility than in those maintained in a sterile, specific pathogen-free environment (Goverman *et al*., 1993). Multiple TLR agonists act as potent adjuvants in the induction of autoimmunity (Hansen *et al*., 2006). The TLR2 agonist PGN is capable of inducing clinical disease in a MOG model of EAE when emulsified in incomplete Freund's adjuvant (IFA), whereas MOG in IFA alone is incapable of inducing disease (Visser *et al*., 2005). In addition, CpG plus LPS can promote MBP-induced EAE in

Lewis rats (Wolf *et al.*, 2007). Activation of antigen presenting cells (APCs) via innate immune receptors can break self-tolerance and trigger the development of autoimmunity even in a genetically resistant strain (Waldner *et al.*, 2004). These studies indicate that innate immune activation in the periphery promotes autoimmune exacerbations in the CNS.

When considering potential links between microbial infections and autoimmunity, it is also necessary to take account of the so-called hygiene hypothesis, in which some infections are posited to keep the immune system balanced, thereby inducing immunoregulation and preventing allergy and autoimmunity (Kamradt *et al.*, 2005).

Activation of the innate immune system within the CNS modulates brain pathology

The expression of TLRs is up-regulated in the CNS during diseases such as EAE (Zekki *et al.*, 2002). It is intriguing that under sterile conditions, innate immune activation within the CNS can modulate disease activity. Indeed, as a consistent feature of different CNS diseases, microglia and astrocyte activation and enhanced expression of TLRs are observed (Kielian, 2006; Farina *et al.*, 2007; Nguyen *et al.*, 2002). This begs the question as to the repertoire of TLR ligands in sterile CNS diseases. On the one hand, it is possible that pathogen-derived TLR ligands could be imported into the CNS by invading immune cells. Indeed, phagocytes containing a disease-promoting TLR/NOD ligand have been observed in the brain during demyelinating disease in primates (Visser *et al.*, 2006). On the other hand, there are also endogenous TLR ligands (see above), that might engage the up-regulated TLRs within the CNS.

In vivo evidence favoring an active role for TLR in sterile CNS diseases has been obtained in several models. Stereotactic axotomy in the entorhinal cortex results in substantial induction of pro-inflammatory cytokines and chemokines. This reaction is markedly reduced in TLR2-deficient mice, (Babcock *et al.*, 2006). Further, encephalitogenic T cells are less efficient in inducing EAE when transferred into TLR9-deficient hosts, suggesting that endogenous host-derived cells aggravate autoimmune inflammation, presumably via an endogenous danger signal (Prinz *et al.*, 2006).

Mice homozygous for a null-mutation of TLR4 develop increased amyloid Abeta deposits. These changes were documented by thioflavine-S staining of fibrillar Abeta aggregates and by demonstration of buffer-soluble and -insoluble Abeta (Tahara *et al.*, 2006). Together, these observations suggest a role of this pattern receptor in modulating the formation of pathogenic deposits in the brain in Alzheimer's disease. Activation of TLR4 expressed by microglia could induce both oligodendrocyte and neuronal injury (Lehnardt *et al.*, 2002, 2003), but may also promote remyelination (Glezer *et al.*, 2006).

In summary, compelling evidence has now accumulated to indicate that the innate immune system shapes CNS autoimmunity, neurodegeneration, and traumatic tissue injury. On the other hand, the biological consequences can be tissue damage or tissue repair.

Adaptive immune reactivity

Adaptive immune reactivity appears late in phylogeny. Jawed fish are the "oldest" vertebrates to use antigen-specific lymphocytes in the fight against microbial infection. Adaptive immune reactivity ideally complements innate immunity. Both response systems share important features: they protect the body against foreign, potentially menacing organisms, but at the same time largely respect the body's own tissues, displaying immunological self-tolerance. However, protection provided by adaptive immunity is more radical; the distinction between foreign and self that is built up is more clear-cut; and, importantly, adaptive immune responses establish immunological memory. One initial contact with a certain bacterium, for example, conditions the adaptive immune system to mount a faster and stronger response following subsequent encounters. Adaptive and innate immune responses use very different cellular and molecular strategies, but it is important to know that they do not operate separately, independent of each other. Both systems are tightly interconnected to orchestrate concerted actions against particular targets.

Adaptive immunity uses stunningly simple and efficacious principles: clonal diversity of immune cells and immune surveillance of the body's tissues. Clonal diversity implies that the immune system forms a large number of individual lymphocyte clones, each one characterized by a membrane receptor that recognizes one distinct antigen. These lymphocytes patrol through the tissues in permanent search of their antigen; they exert immune surveillance. The actual immune response, however, unfolds in the secondary lymph organs, lymph nodes and spleen. It

culminates in the generation of effector mechanisms that are aimed at removing or neutralizing the foreign agent, the antigen, in question.

The adaptive immune response employs three main cellular components. The T lymphocytes are the principal patrollers, migrating through the body and gathering information. During an immune response, they mature either to effector cells that directly attack the antigen, or to regulatory cells that enhance or reduce the activity of the ongoing response. The B lymphocytes, in contrast, are less involved in immune surveillance, but instead they produce humoral antibodies that can bind to antigenic structures and tag them for destruction by macrophages and the complement system.

The third principal player in the immune response is the dendritic cell (DC), a cell type only discovered in the 1970s. The DCs play a pivotal role interconnecting innate and adaptive immune responses. They have the unique ability to sequester protein, to process it, and to ultimately display particular peptide fragments to specific T lymphocytes. The T lymphocytes, in contrast to B lymphocytes, have surface receptors that recognize only *processed* antigen, i.e. antigenic peptide fragments bound to proteins of the major histocompatibility complex (MHC). In addition to serving as the classical APCs to T lymphocytes, DCs are extremely sensitive to stimuli provided by innate immune responses. Thus, DCs display on their membrane PRRs at high density, and following stimuli, they become activated, a process that further intensifies processing of antigen and its presentation to local T lymphocytes.

Does adaptive immunity protect the CNS? Do the main rules of adaptive immune responses apply to the brain and spinal cord? At first glance, this concern may appear moot, but it is not. It should be kept in mind that, until recently, the CNS was deemed exempt from adaptive immune reactivity. After all, the CNS is secluded from circulating immune cells by a tight endothelial BBB, and, in addition, structures critically required for immune responses are missing in the healthy CNS (Wekerle *et al.*, 1986). Indeed, the normal CNS keeps immune reactivity to a minimum. It provides a milieu hostile to immune cells and their function. Normal CNS tissue fails to produce important MHC products, cell adhesion molecules, chemokines and cytokines that are required for successful immune responses. Under a number of pathological conditions, however, the BBB becomes more permeable, and CNS cells can be induced to de novo express molecules relevant to the adaptive immune response: the CNS tissue milieu turns from immune-hostile to immune-friendly (Wekerle, 2006).

Immune cells in the CNS – T cells

Inflammatory cells invading the CNS were initially described by pathologists, notably in the context of CNS infections and tumors, but also in neurodegenerative disease. Inflammatory infiltrates are particularly notable in active lesions of MS, a CNS disorder, which is not caused by any known specific infectious agent, and which, for several reasons, is thought to be the consequence of an autoimmune attack (Lassmann and Wekerle, 2006).

These observations raise questions of clinical as well as biological importance. What are the conditions that lure inflammatory cells into the normally secluded CNS tissue? How do these cells interact with local CNS components both in health and disease? And, more generally, if there is any immune surveillance of the CNS, how is it organized? Pertinent answers emerge from studies of experimental autoimmune encephalomyelitis (EAE), an animal model of CNS autoimmunity.

T cell migration through the BBB

The endothelial cells lining the microvessels within the CNS are distinct from the endothelia of other organs. They are specialized to form a vascular lining impermeable to most blood macromolecules and cells. The BBB endothelia are interconnected by complex arrays of tight junctions. The few blood-borne molecules required by CNS cells pass through the endothelia by active transport systems (Abbott *et al.*, 2006). Among circulating blood cells, only a few activated lymphocytes and some macrophages seem to be able to pass through the BBB. In health, the BBB inner surface lacks most of the structures required by circulating leukocytes to attach and to navigate through the vessel wall (Engelhardt and Ransohoff, 2005).

The BBB endothelia are, however, readily activated and rendered receptive for leukocytes following several modifications, either initiated systemically, or within the CNS tissue (Ransohoff *et al.*, 2003). In vitro studies have shown that inflammatory stimuli like bacterial lipopolysaccharide (LPS), or cytokines induce BBB endothelia to form cell adhesion molecules or chemokine mediators required for lymphocyte transmigration (Wong and Dorovni-Zis, 2000; Shukaliak and Dorovni-Zis, 2000). In vivo application of these stimuli

enhances leukocyte traffic into the CNS in a similar way (Piccio *et al.*, 2002). Importantly, however, leukocyte immigration into the CNS is not only triggered consequent to inflammation, but also in the context of neurodegenerative disease (Ransohoff and Tani, 1998). Loss of neuronal function leads to the production of cytokines that seem to recapitulate much of the events that enhance leukocyte infiltration in microbial infection and autoimmunity (Raivich *et al.*, 2003).

Obviously, interactions between recirculating immune cells and (activated) immune cells are of paramount clinical importance. Such interactions are beneficial in infectious diseases or tumor formation, where the pathogenic process could be limited by incoming immune cells. In these situations, immune cell immigration should be supported therapeutically. In contrast, in cases of anti-CNS autoimmunity (as in MS), invading immune cells are pathogenic. The results of recent trials of anti-integrin α4 antibody natalizumab therapy in MS illustrate this point. These antibodies are reputed to mask a cell adhesion molecule involved in guiding activated T cells through the BBB, and they reduce the number of new relapses and of radiologically demonstrable CNS lesions impressively (Miller *et al.*, 2003). Unfortunately, a small number of patients developed progressive multifocal leukoencephalopathy caused by reactivated JC virus, either dormant in the CNS or transmitted from the periphery (Ropper, 2006). Reactivation might have been caused by compromised anti-microbial immune surveillance, a detrimental side effect of antibody treatment, or alternatively by release of JC virus-containing cells from the bone marrow (Ransohoff, 2007).

Antigen presentation and immune reactivity within the CNS

Previously, we mentioned that, under *normal* conditions, neurons and glial cells within the CNS milieu fail to produce and expose MHC determinants, cell adhesion molecules and soluble mediators necessary for productive immune reactivity. However, we also stressed that CNS cells are by no means unable to produce such molecules. The CNS cells can be induced in varying degrees to produce "immune" genes, and the inducing signals can be provided in the course of processes as diverse as virus infection, tumor growth or, quite surprisingly, neuronal degeneration.

There is a hierarchy of potential APCs within the CNS milieu. Clearly, the most efficient APCs are derived from bone marrow-derived progenitors: resident microglia, as well as macrophages infiltrating from circulating blood. Other glial cells, namely astrocytes and oligodendrocytes, or neurons, may be able to present antigens in the effector stage of immune responses (Wekerle, 1994), and thus can be recognized as targets by effector lymphocytes; their capacity to trigger de-novo immune reactions seems, however, to be confined to discrete circumstances. Under inflammatory conditions, microglia are readily induced to express both MHC class I and class II and to present antigens to T cells. Cytokine-stimulated astrocytes are far less competent in presenting antigens than microglia (Aloisi *et al.*, 2001). Inducibility of MHC class I in neurons is very strictly regulated and full-blown expression of MHC class I on the neuronal cell surface was only observed after treatment of electrically paralyzed neurons with interferon-gamma (Neumann *et al.*, 1995, 1997b).

Autochthonous CNS cells are inducible, facultative APCs, but does the CNS harbor any *professional* APCs, that is DCs? This long-standing debate has, apparently, been answered in the affirmative. Professional APCs are capable of delivering additional co-stimulatory signals to T cells that serve to stimulate the full activation program of naive T cells. Tissue-resident professional APCs are not detected in the normal CNS parenchyma. However, macrophages are activated and substantially increase in number during autoimmune inflammation (Lassmann *et al.*, 1993). Dendritic cells are recruited into the CNS and mainly accumulate in the perivascular area of overt inflammatory foci during EAE (Serafini *et al.*, 2000) and cerebral toxoplasmosis (Fischer *et al.*, 2000). These CNS-associated DCs are credited to play a central role in the pathogenesis of neurological immune diseases; their perivascular location means that they are the first cell type encountered by T cells passing through the microvascular BBB. Presentation of local autoantigen could serve as a guidance signal directing the autoimmune T cells into their target destination (Greter *et al.*, 2005).

Beyond this signaling, DCs, as classical professional APCs, have the ability to recruit naive T cells. Within the CNS, DCs would be the only APCs able to take up and present local autoantigens and thus to activate T cells of specificities other than the original effector cells. Dendritic cells isolated from the brains of mice affected with inflammation contain CNS material and activate a complement of T cells reactive to an extended range of myelin autoantigens (McMahon

et al., 2005). Furthermore, recent observations indicate that intracerebral DCs drive naive myelin-specific T cells into the Th17 lineage, the lineage enriched for pathogenic effector T cells (Bailey *et al.*, 2007).

Is there a communication between the CNS and the peripheral immune system? While there is no doubt that inflammatory cells, at least under favorable conditions, are able to cross the BBB and to enter CNS tissue, there is, however, much less evidence of migration in the opposite direction, from within the CNS to the periphery. While emigration of T (and B) cells has never been observed, there are at least circumstantial indications that the extrusion of CNS antigenic material occurs, either by leakage of subcellular material or via carriage within phagocytes. After intracerebral injection, protein markers were seen in local lymphatic organs, mostly the deep cervical lymph nodes (Bradbury and Cole, 1980; Cserr *et al.*, 1992). Moreover, cervical lymph nodes seem to be involved in the EAE response targeted to particular areas of the cerebral cortex (Phillips *et al.*, 1997). More recent studies followed the migration of DC-like cells from the CNS to surrounding lymphoid organs (Hatterer *et al.*, 2006). Indeed, myelin autoantigen is also demonstrable in human cervical lymph nodes from patients with MS (Fabriek *et al.*, 2005) and in primates with EAE (De Vos *et al.*, 2002).

B cells in the CNS

If the healthy CNS fails to provide a favorable milieu for T cells, the same is true with regard to B cells. However, as with T cells, there are pathological conditions that favor the entrance of B cells into the CNS, and their persistence in this location.

In normal CNS tissue, some B lymphocytes can be found, but they are rare (Anthony *et al.*, 2003). B cells are, however, commonly found in inflammatory lesions, such as in MS plaques (Meinl *et al.*, 2006). Intriguingly, there seems to be a certain propensity for B cell lymphomas to expand in brain and spinal cords. Primary lymphomas arising within the CNS are mostly of B cell, and only exceptionally of T cell, origin (Iwamoto and DeAngelis, 2006).

The best-known consequence of B cell activity within the CNS is the appearance of B cell products, immunoglobulin distributed as oligoclonal bands, in the cerebrospinal fluid of patients with MS, or microbial infections. At least some of these immunoglobulins are actively produced within the CNS tissue, or the enshrouding leptomeningeal membranes. In the case of (viral) infections, these antibodies may be specific for the infectious agent. In MS, however, it has been difficult to assign specificity to these antibodies. There is evidence that some of the antibodies bind with low affinity to myelin proteins, but direct evidence of a positive role in the pathogenesis of MS is still elusive. Nevertheless, studies of the primary structure of CSF immunoglobulins indicate a positive, T cell-driven process controlling their production, with evidence of somatic mutation and immunoglobulin class switches.

The intricacies of B cell biology within the CNS are still to be unravelled. For example, it is uncertain whether B cells crossing the BBB respond to either specific signals emanating from the CNS cells proper, or from locally responding T cells, or, whether they merely migrate with invasive T lymphocytes as passive fellow travelers. Most of our present knowledge is derived from experimental models such as EAE. Although most variants of EAE are primarily T cell-mediated autoimmune diseases, there are significant B cell contributions. The B cells act in several stages of the disease. In the emerging autoimmune response, B cells can pick up soluble autoantigen, process it and present it to specific T cells. Then, via the specific repertoire of cytokines that B cells produce, they can help to shape the particular T cell phenotype required to attack the CNS target tissue. Finally, in the effector phase, B cells can act via their autoantibody products, which can bind to CNS membrane structures, and with the help of complement and/or macrophages, can initiate tissue destruction (Schnell *et al.*, 1997).

The CNS milieu for B cells

We have previously emphasized that, in general, the CNS tissue provides a microenvironment adverse to immune cell survival and local immune responsiveness. This statement was mainly directed to T cells and their cooperating cell partners. Interestingly, B cells may find the CNS milieu more hospitable, as suggested by recent investigations (Uccelli *et al.*, 2006). Astrocytes are able to produce and release B cell activating factor (BAF), an essential soluble mediator supporting survival and reactivity of B lymphocytes (Krumbholz *et al.*, 2005). This may explain why some B cells persist over extended periods of time within the CNS.

There is good evidence of structures involved in B cell migration into or within the CNS. It also appears that the CNS parenchyma, either resting or activated, can supply soluble signals that could guide recirculating

B cells through the BBB. For example, the chemokine CXCL12 is detected in both the microvascular wall and in adjacent astrocytes (Krumbholz *et al.*, 2006). In contrast, another B cell chemokine, CXCL13, appeared mainly restricted to inflammatory infiltrates. The level of CXCL13 expression was correlated to the number of infiltrating B cells (Krumbholz *et al.*, 2006).

Studies of an in vitro model of the BBB suggest that the motility of human B cells is similar to that of T cells, and that the two major lymphocyte sets display similar profiles of adhesion molecules and chemokine receptors (Alter *et al.*, 2003), but that they use distinct combinations of proteases to open tissue barriers (Bar-Or *et al.*, 2003).

B cell-containing lymphoid tissues ("ectopic lymphoid tissues")

Transient immune responses take place within the preformed, specialized secondary lymphoid organs, lymph nodes, spleen, gut and bronchus-associated lymphatic tissues. In chronic responses, such as in chronic infection and autoimmune responses, lymphatic tissue can be formed de novo in the vicinity of the actual disease process/target tissue. In rheumatoid arthritis, for example, large lymphatic infiltrations forming germinal centers are typically noted in the inflamed synovial pannus. In Sjögren's syndrome, similar infiltrates change the structure of the lacrimal and salivary glands, and in Hashimoto's thyroiditis they dominate the thyroid (Hjelmström, 2001).

The newly formed lymphoid tissue associated with target organs could contribute to the course and character of the ongoing autoimmune response. Autoantigen produced in the vicinity may enter these lymphoid areas, and there foster the ongoing cellular response. Presentation of these determinants can be expected to activate and recruit pathogenic T cells, and, in addition, give rise to the production of humoral autoantibodies (Aloisi and Pujol-Borrell, 2006).

Germinal center-like formations are common in the avian CNS, where they have been repeatedly described in the pineal gland (Cogburn and Glick, 1981). They have not been noted in healthy mammalian CNS tissue, but occur in a subgroup of patients with MS. In 1979, Prineas described thin-walled microvessels reminiscent of lymphatic vasculature embedded in germinal center-like lymphocyte aggregates. These tissues were located in perivascular areas (Prineas, 1979). An important, and baffling, but not entirely unpredictable, observation was the description of follicle-like organized tissue in the CNS of certain MS cases (Serafini *et al.*, 2004), and these may associate with cortical pathology (Magliozzi *et al.*, 2007). Immunocytochemistry revealed a composition of B cells, T cells, plasma cells and most intriguingly, follicle dendritic cells, the signature cells of differentiated germinal centers (Serafini *et al.*, 2004). These follicle-like structures were noted predominantly within the leptomeningeal membranes, in perivascular areas, and Virchow–Robin spaces, but not within the parenchyma proper (Magliozzi *et al.*, 2007).

Formation of fully differentiated lymphoid tissue involves members of the TNF family of genes. The cytokine, lymphotoxin-alpha (LT-α), LIGHT and other mediators induce the formation of a milieu that promotes the establishment of ordered lymphatic tissues, among them prominently, germinal centers (Ware, 2005). In a chronic-relapsing model of mouse EAE, neutralization of LT-β by a recombinant antagonist protein curbed ongoing disease and at the same time prevented formation of leptomeningeal lymphoid aggregates indicating a central role for LT-β in this activity (Columba-Cabezas *et al.*, 2006). Other cytokines, such as IL-7, also contribute (Meier *et al.*, 2007).

Intrathecal production of immunoglobulins

One hallmark of CNS inflammation, either induced by microbes or autoimmunity, is the formation of oligoclonal immunoglobulin bands in the cerebrospinal fluid (CSF). These immunoglobulins are distinct in their electric charge. As antibody products of a limited number of plasma cells resident within the confines of the CNS they can be readily separated electrophoretically. In contrast, serum immunoglobulins, which are produced by millions of diverse plasmablasts and plasma cells that are present in the peripheral immune repertoire, overlap to form a continuum.

Oligoclonal CSF immunoglobulins in CNS infections commonly include bands that bind structures of the relevant infectious agents such as measles virus in subacute sclerosing panencephalitis (SSPE) (Mehta *et al.*, 1982) and *Borrelia burgdorferi* in neuroborreliosis (Murray *et al.*, 1986). Antigen specificity of infection-associated Ig bands readily indicates their association with an anti-microbial B cell response.

Less clear, however, is the nature and origin of CSF bands in MS. Intensive studies over the past decade

were not able to clearly assign antigen specificity to these antibodies. The claim that the oligoclonal bands in MS are specific for *Chlamydia pneumoniae* (Sriram *et al.*, 1999) was not confirmed in other studies (Derfuss *et al.*, 2001). Binding of oligoclonal bands to EBV, a common human virus of the herpes group, was reported (Cepok *et al.*, 2005), but is not yet confirmed in other studies. These shortcomings led to the contention that CSF immunoglobulins have no role in the actual autoimmune disease process. They might rather represent non-specific antibodies generated in the CNS due to bystander processes. Specific antibodies would be absorbed within the target tissue, with non-specific antibodies leaking out into the CSF.

While oligoclonal bands occur in the CSF of both MS patients and patients with an encephalitis with a known infectious agent, one important difference between the intrathecal Ig production of MS patients and other inflammatory neurological disease (OIND) patients has been known for many years: whereas MS patients typically display an intrathecal immune response against many different common pathogens such as measles virus, rubella virus, and varicella-zoster virus, as well as *Chlamydia pneumoniae* and HHV-6, OIND patients do not (reviewed in Anthony *et al.*, 2003). This polyspecific anti-pathogen Ig does not correspond to the major OCB in the CSF and is considered a bystander reaction (Measles–Rubella–Zoster reaction), which can be detected in about 90% of MS patients. The reason for this polyspecific Ig response in MS is unclear; it probably does not simply reflect a consequence of long-lasting disease, since it is typically present at the beginning of MS and is even used as a diagnostic criterion in some clinics. The polyspecific intrathecal Ig response in MS might indicate an environment that promotes enhanced B cell activity long before the clinical disease starts, and could also reflect the individual's history of infections (Anthony *et al.*, 2003).

Immunoglobulins in the brain tissue of MS patients

Early studies eluted antibodies from MS plaque material and identified their oligoclonal distribution (Mehta *et al.*, 1981; Glynn *et al.*, 1982). The identification of antigen specificity of immunoglobulins dissociated from CNS lesions remains a challenge to the present day. While one study initially found binding to native MOG in 50% of samples from MS-derived autopsy material, but not in non-MS control samples (O'Connor *et al.*, 2005), the same group using more elaborate technology restricted this specificity to a few cases of acute disseminated encephalomyelitis (ADEM), but did not find antibodies to MOG in the serum in classic MS (O'Connor *et al.*, 2007).

In one particular pattern of MS plaques, immunoglobulin bound to myelin debris along with activated complement is the structural hallmark suggesting an active participation of autoantibodies in the pathogenic process (Luchinetti *et al.*, 2000). Furthermore, one group suggested that at least some of these bound antibodies are specific for MOG autoantigen (Genain *et al.*, 1999), a claim, which, however, waits to be formally confirmed by independent studies.

Analysis of Ig rearrangement in the CNS

Frustrating as the search for the target autoantigen may have been, the study of the molecular nature of Ig transcripts in CSF provided important insights into the pathogenesis of MS.

In order to appreciate these data, it should be kept in mind that antigen-driven B cell responses are the result of a complex interaction between helper T cells, mainly of $CD4^+$ lineages, and specific B cells. Both lymphocyte sets recognize the same antigen, through distinct epitopes via distinct mechanisms. Activated T cells ultimately drive B cells to sharpen their antigen specificity via somatic mutation of their immunoglobulin hypervariable regions (complementarity determining regions) and trigger the molecular switch from "primitive" IgM to "effector" isotypes (IgG, IgE, or IgA) (Ahmed and Gray, 1996).

Qin *et al.* used PCR amplification to study CSF-derived B cells and established a hierarchy of sequences that suggested somatic mutation of expanding B cell clones, data that have been supported by other groups. This likely represents a T helper cell-driven B cell response (Qin *et al.*, 1998; Owens *et al.*, 1998; Baranzini *et al.*, 1999; Colombo *et al.*, 2000). More recently, investigators have turned to the study of single B cells, mostly isolated by cytofluorometric cell sorting from CSF (Ritchie *et al.*, 2004), an approach that ultimately allows cloning and expression of paired immunoglobulin, i.e. H and L chains from the same individual B cell (Haubold *et al.*, 2004). Expression of CNS-associated immunoglobulins as recombinant Fab or Fv fragments have been used lately to search for relevant target autoantigens. One

study identified polyreactive myelin binding immunoglobulins (Lambracht-Washington *et al.*, 2007), while another pointed to enzymes of the glycolytic pathway as targets that are expressed in neural compartments (Kolln *et al.*, 2006).

B cell models?

There is a deficiency of valid models to study the role of B cells in CNS immune reactivity. In EAE, classical experiments by Linington and colleagues explored the function of anti-myelin autoantibodies by co-transferring relevant MAbs along with encephalitogenic effector T cells (Schluesener *et al.*, 1987; Linington *et al.*, 1988). In these paradigms, the T cells attack the brain white matter and thereby open the BBB to permeation of co-transferred MAbs. This strategy was helpful for studying the role of B cell-derived autoantibodies in the effector phase of an autoimmune attack, such as the interaction of membrane-bound MAbs with complement factors (Piddlesden *et al.*, 1993).

However, the role of B cells in CNS autoimmunity is more complex. The B cells can act as APCs, concentrating and presenting myelin autoantigen to T cells. They also secrete cytokines that influence the character of immune responses. One approach to gain insight into these complexities is the use of transgenic mice with a B cell repertoire dominated by B cells specific for myelin autoantigens; for example, MOG. Litzenburger *et al.* replaced the immunoglobulin J region by the rearranged gene encoding the H chain of the original anti-MOG MAb 8–18C5 and found that about 30% of all mature B cells produced immunoglobulin binding to MOG (Litzenburger *et al.*, 1998). In double-transgenic mice, these B cells actively cooperate with $CD4^+$ T cells expressing MOG-specific T cell receptors to bring about a spontaneous autoimmune disease involving the optic nerves and spinal cord as primary targets (Bettelli *et al.*, 2006; Krishnamoorthy *et al.*, 2006).

Conclusion

Although innate and adaptive immunity are mechanistically distinct, they reflect components of an integrated response to either exogenous or endogenous danger signals. Much dogma concerning the nature of the CNS immune response and how it interacts with immune activation in the periphery has broken down in recent years. However, the exact nature of these interactions and, in addition, how T and B cells cooperate to induce CNS autoimmunity remain ill-defined. It will be especially important to understand the principles governing these interactions if we are to develop selective strategies that can inhibit CNS autoimmunity but which do not compromise antimicrobial immune surveillance, a major challenge for future research.