[Frontiers in Bioscience 7, d427-438, Feburary 1, 2002]

IMMUNE RESPONSE INDUCTION IN THE CENTRAL NERVOUS SYSTEM

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1. ABSTRACT

The primary function of the immune response is protection of the host against infection with pathogens, including viruses. Since viruses can infect any tissue of the body, including the central nervous system (CNS), it is logical that cells of the immune system should equally have access to all tissues. Nevertheless, the brain and spinal cord are noted for their lack of immune presence. Relative to other organ systems, the CNS appears immunologically privileged. Furthermore, when immune responses do occur in the CNS, they are frequently associated with deleterious effects such as inflammatory and/or demyelinating pathology. This article will review the molecular and cellular dynamics of immune responses in the CNS, with particular emphasis on autoimmune inflammation, as has been studied in the authors' laboratory.

2. IMMUNE RESPONSES IN THE CENTRAL NEROUS SYSTEM

Two major aspects that influence immune responses in the central nervous system (CNS) are the relative lack of major histocompatibility complex class I and II (MHC I and II) expression, and restricted access to immune cell entry. The former mediates against recognition of antigen by T lymphocytes, especially by CD4-positive T cells, which recognize antigen associated with MHC II, whose expression in CNS is particularly sparse. Immune responses in the CNS include those which are protective, or whose etiology may be assumed to relate to host-protection, and those which are directed against CNS antigens (autoimmune). Because host-protective responses may also induce deleterious inflammation and encephalitis, the distinction between the two can become blurred.

2.1. Protective responses

Immune responses to viral infections of the CNS, like those elsewhere in the body, are dominated by CD8-positive, MHC I-restricted, T cells. These kill infected cells by cytotoxicity, and induce associated inflammatory responses through expression of interferon-gamma, which is also anti-proliferative and so inhibits viral replication. Theiler's Murine Encephalitis Virus (TMEV) infects oligodendrocytes, the cells which produce myelin (1). Virally-infected oligodendrocytes express MHC I. The CD8-positive T cell response against TMEV is associated with damage to and loss of myelin, and resultant encephalitis (1). Interestingly, in mice which lack CD8-positive T cells due to lack of beta2-microglobulin and MHC I, virus infection of oligodendrocytes results in cell death and loss of myelin, but without the usual

inflammatory response, encephalitis does not ensue (2). The TMEV mimics in some respects the clinical pathology of Murray Valley Encephalitis (MVE), which results from neuronal infection with a mosquito-borne virus. MVE pathology is associated with neutrophil infiltration to the CNS (3).

The Borna disease virus, an RNA virus, induces degenerative pathology in CNS of Lewis rats, which is accompanied by CD8-positive and CD4-positive T cell infiltration. The CD8-positive T cells are both the principal effectors of virus clearance – although the virus is never completely cleared and a noncytolytic, persistent, chronic infection ensues - and causative agents of encephalomyelitic pathology (4).

The spirochete *Borrelia burgdorferi* is the causative agent of Lyme Disease, a chronic arthritis that results from cross-reaction between spirochete proteins and collagen. *Borrelia* may also infect CNS tissue, and cross-reactivity with myelin proteins results in Lyme Encephalitis. CD4-positive T cell infiltration and demyelination feature in this disease, which shares many pathological features with Acute Disseminated Encephalomyelitis (ADEM; ref. 5). In both cases, cross-reactivity, or Molecular Mimicry, leading to autoimmunity (6-8), is incidental to the primary goal of the immune response, which is eradication of the infectious organism.

Cells in the CNS are also prepared to respond to bacterial infection. Anti-bacterial immune responses in CNS are primarily those against Haemophilus influenzae, Neisseria meningitidis and Streptococcus pneumoniae. Meningitis-associated immune responses are profoundly inflammatory and frequently associated with transient encephalitis (9). As the term implies, immune responses in meningitis do not usually attack myelin or induce encephalomyelitis. During acute meningitis, increased CNS expression of CD14, which binds bacterial lipopolysaccharide, is detectable by increased levels of soluble CD14 in cerebrospinal fluid (10). Constitutive expression of both CD14 and Toll-like receptor 4 mRNA in CNS, especially in areas devoid of blood brain barrier (e.g., circumventricular organs), may establish innate CNS immunity to bacterial infections (11, 12). Similarly, constitutive and enhanced expression of complement proteins in the CNS provides for an immediate response to invading pathogens (13).

2.2. Autoimmune responses

Autoimmune reactivity towards the CNS is generated experimentally by immunization of animals (usually but not exclusively rodents) with myelin proteins (14). Immunization with non-myelin CNS proteins can induce autoimmune reactivity and infiltration, but has not to date been successful in inducing autoimmune disease (15). Self-tolerance considerations dictate that aggressive immunization, with at least one if not two adjuvants, is needed for induction of overt disease. One of the adjuvants commonly used, pertussis toxin, is considered to play a role in enabling access of activated immune cells to the CNS via a compromised blood-brain barrier (16). Complete Freund's Adjuvant includes heat-killed *Mycobacterium tuberculosis*, which is speculated to be effective through its ability to induce an inflammatory, T-helper1 (Th1) type of T cell response (17).

The Th1 responses are dominated by production of the cytokine interferon-gamma (IFN-gamma), which is either induced by the myeloid cell cytokine interleukin-12 (IL-12), or predisposition to IFN-gamma production is selected from a T cell population by IL-12 (18). Either way, the Th1 antigen-specific CD4-positive T cell is specialized through adhesion molecule and chemokine receptor expression for extravasation from blood to inflamed tissues, and is implicated in the induction of CNS autoimmune inflammation in most models (18).

Induction of experimental autoimmune encephalomyelitis (EAE) is absolutely dependent on the induction of a Th1 CD4-positive T cell response to a myelin antigen (19, 20). The only exceptions that have been described are genetically engineered mice which are restricted to recognition of myelin antigens, and in which the opposing cytokine profile, T-helper2 (Th2), is deliberately induced (21). This relatively non-physiological situation serves to demonstrate that Th1 cytokines are not essential for disease, an observation that can be made in other systems (*vide infra*).

Once an anti-myelin CD4-positive T cell response has been induced, then the progression of disease may be effected by other cell types, but the data strongly suggest that the CD4-positive T cell is critical for induction, and in almost all situations, for direction of subsequent immune inflammation. This was the case even in a recent study that showed that CD8-positive T cells could effect EAE. The authors concluded that the role of CD4-positive T cells in initiating disease remained paramount, even though they could transfer disease with CD8-enriched T cell populations (22). Nevertheless, one must consider whether a distinction might be drawn between induction of an immune response in the periphery, and the effect of that response in a target organ. Whether the ability of activated CD8-positive T cells to effect disease could be separable in time from their induction, at sites distal from the CNS, is a question with relevance to many clinical pathologies, including Multiple Sclerosis (MS).

The pathology of experimental autoimmune inflammatory disease in CNS varies of course with strain and species of animal, choice of myelin antigen and other variables, but there are features in common between the various systems. Leukocytic infiltrates are invariably found in CNS, whether parenchymal (not common), perivascular or meningeal. Loss of myelin integrity or frank demyelination, whether widespread or local to infiltrates, are more common than not. A generalized inflammation of the vasculature is usually seen, especially if disease was induced by immunization with adjuvant, but also if disease was adoptively transferred by systemic injection of activated T cells. The composition of leukocytic infiltrates varies depending on the circumstances of disease, but

almost always includes T lymphocytes, themselves usually (but not always) dominated by CD4-positive T cells. Macrophages are commonly seen, the proportion of neutrophils varies from system to system, and activated CNS glia (astrocytes, microglia) - are always described (19).

2.3. Other responses

Other circumstances where immune infiltration of the CNS occurs include reperfusion after cerebral ischemia. Immune infiltration to ischemic infarcts is not predicated on specificity to CNS antigens. Infiltrates are dominated by neutrophils and activated macrophages (23).

In Cerebral Malaria, parasite (*Plasmodium falciparum*) accumulation in infected erythrocytes in cerebral vessels leads to breach of the blood-brain barrier, and intrusion of macrophages and neutrophils, with accompanying glial reactivity and cytokine production (24). The inflammatory cytokine, tumour necrosis factor (TNF), is particularly implicated in the pathology of cerebral malaria.

Alzheimer's disease (AD) is not considered either autoimmune or immune-involving. Nevertheless, microglial activation is a recognized aspect of AD pathology, which has parallels to myeloid cell involvement in immune responses in CNS. Furthermore, vaccination against AD pathology using beta-amyloid peptides has successfully abrogated aspects of AD-associated pathology in mice, arguing for immune access if not surveillance, or its deficit, as components of AD (25-28).

The above by-no-means complete list of circumstances in which immune cells infiltrate and induce pathology in CNS is intended to both document such events, and to highlight their relative rarity. The CNS is not noted for its content of immune cells or molecules, and is regarded as immune privileged as a consequence.

3. FACTORS THAT CONTRIBUTE TO CNS IMMUNE PRIVILEGE

The principal factor that contributes to the relative immune privilege of the CNS is the blood-brain barrier (BBB). Unlike endothelia in blood vessels elsewhere in the body, with their fenestrated endothelia that permit unrestricted access of cells and macromolecules to those tissues, cerebral capillaries have tight junctions and form part of a complex barrier to CNS entry. The endothelium proper is complemented by a perivascular space and a basement membrane that separate it from the CNS parenchyma, and which collectively constitute the extracellular matrix (ECM) which must be penetrated by cells that would extravasate at these locales (20, 29). Apposition of astrocyte end-feet to the basement membrane adds another dimension to the BBB. Microglia, myeloid bone-marrow-derived cells that may be considered brain macrophages, are also in close contact with the parenchymal face of the BBB. Both microglia and astrocytes constitute a reactive component of the BBB that may respond to intra-CNS events (29).

Classical experiments using systemic administration of dyes showed the effectiveness of the BBB in restricting molecular access to the CNS - the more modern version of this experiment uses large tracer molecules such as horseradish peroxidase (30). The general absence of immune cells in CNS has prompted suggestions that cellular entry is also restricted. However, restriction is relative, and as will be discussed, leukocytes can and do enter the healthy CNS. It should be noted that all CNS/blood interfaces are not equally impermeable. At sites such as the median eminence, choroid plexus, area postrema and caudal spinal cord, interchange between blood and cerebrospinal fluid (CSF) occurs quite readily (29). It is noteworthy that these are not noted as sites of autoimmune or host-protective infiltration. That may relate to their relative lack of MHC-expressing macrophages/microglia (20, 31, 32).

There is almost total absence of MHC expression in CNS. In particular, healthy brain and spinal cord are almost devoid of MHC II, which would seem to disallow induction of CD4-positive T cell responses. The only cell type which can be shown to express MHC II at any level in the healthy CNS, and which are prominent among MHC II-expressing cells when the CNS is inflamed, are microglial cells (32, 33). This has focused interest on microglia as potential antigen-presenting cells and inducers or amplifiers of immune responses in the CNS.

Other factors that contribute to relative immune privilege of the CNS include that CNS parenchymal tissue may represent an immunosuppressive environment, and that CNS microglia have been reported to be incapable of inducing a full-blown T cell response, compared to splenic macrophages and other peripheral antigen-presenting cells (34, 35). A role of Fas-ligand/CD95L in promoting an immunosuppressive environment has been demonstrated in the testis and retina (36, 37), and proposed for the CNS. Constitutive expression of this molecule enables cells within a tissue or tumor to induce Fas/CD95-expressing leukocytes to apoptose, thus conferring protection to the tissue (38). However, constitutive Fas-L expression in CNS has not been demonstrated (39), and Fas-L deficient mice are not especially prone to CNS autoimmunity (40).

4. HOW DO IMMUNE CELLS ENTER THE CNS?

Entry of blood-derived cells to the CNS is mediated by the same fundamental mechanisms that apply to extravasation from blood to other tissues. Thus, leukocytes trafficking in cerebral capillaries engage in low affinity interactions with endothelial cells, via ligation of adhesion molecules such as L-selectin by counter-ligands on endothelia (17, 41). These low affinity interactions convert fast traffic to a 'rolling adhesion', which slows leukocytes and allows other, more high affinity interactions to occur. Higher affinity interactions include those between alpha-beta integrins on leukocytes and their (usually immunoglobulin superfamily) counter-ligands on endothelia (17, 41). Critical to the role of antigen or infection in directing events is the fact that expression of adhesion ligands and counter ligands is itself promoted or

enhanced by inflammation or prior interaction with antigen-presenting cells. Thus, for instance, T cells that were recently activated by antigen recognition, and endothelia near or at sites of infection, are favoured participants in these immune endothelial interactions (17). One factor contributing to this is the increase in affinity of integrins for their ligands, when the integrin-expressing T cell has received a signal via its antigen receptor (42, 43).

Adhesion ligands are not the sole determinant of immune-BBB interactions. Chemokines are small, usually soluble molecules that are secreted by activated astrocytes, microglia and endothelia, as well as by leukocytes themselves (44-46). They are classified into 4 families on the basis of amino-terminal cysteine residue homologies. There are corresponding homologous families of receptors, with strict intra-family chemokine-receptor fidelity, although shared receptor use within families is fairly common. Chemokine receptors are 7-transmembrane chain complexes, and transduction of signals leads to increased affinity of cell surface integrins, alteration of adhesion molecule expression, and expression and secretion of cytokines (47).

As their name implies, chemokines subserve a chemo-attractant role. Cells expressing specific receptors migrate up concentration gradients of chemokines, sometimes facilitated by association of chemokines with ECM molecules. Thus, chemokine production at an inflammatory site can direct leukocyte traffic to that site. This direction is not random, but shows lineage specificity for leukocytes (44, 47).

The alpha or CXC and the beta or CC chemokines are most numerous, and are associated with inflammation and immune responses. Although functional associations are not watertight, alpha chemokines are generally associated with myeloid/granulocyte responses, and the beta chemokines with T cell/macrophage responses. In mice that lack the cytokine IFN-gamma, alpha chemokines are produced and the beta chemokine production normally associated with EAE does not occur, resulting in a switch from macrophage to neutrophil-dominated infiltration to CNS (47, 48).

Matrix Metalloproteinases (MMP's) are a family of serine proteases that digest components of the ECM, and so are implicated in leukocyte entry to the CNS. Expression of selected MMP's (MMP-9, MMP-12) is elevated in CNS during EAE and MS, and MMP blockade or inhibition can inhibit EAE[49]. Care must be taken in interpreting such findings, because MMP's act on other substrates that may influence immune responses. For instance, MMP-7 and MT4-MMP are implicated in cleavage of membrane-bound TNF to release soluble cytokine, and MMP's may exert neurotoxicity (reviewed in ref. 50). Nevertheless, it is widely believed that ECM-digestion is a major component of MMP's role in immune responses in the CNS.

4.1. Entry of naïve lymphocytes to healthy CNS

Evolution of thinking on CNS immune privilege began with the realization that leukocytes could enter the CNS. The rolling-adhesion model for leukocyte endothelial interaction allowed qualification to activated T cells, and later to naive T cells if the BBB endothelium or underlying tissue were inflamed or elaborating chemo-attractant signals (31, 41). In support of this, activated ovalbumin (OVA)- or PPD-specific T cells were shown in a number of studies to enter either uninflamed or inflamed CNS (reviewed in ref. 20). More recent data takes this evolution a step further, showing that naive, or resting T lymphocytes, not only can enter the CNS, but may be found in relatively large numbers (51, 52).

Two recent studies have contributed to this paradigm shift. Krakowski and Owens (52) showed that ovalbumin-specific T cells accumulated in large numbers, equivalent in proportion to encephalitogenic myelin basic protein-specific T cells, in the CNS of mice with EAE. The OVA-specific T cells were shown to express a naive phenotype and not to express the inflammatory cytokine IFN-gamma. It was assumed that compromise to the BBB by infiltration of MBP-specific T cells facilitated entry of naive OVA-specific cells (52). However, Brabb *et al.* (51) showed that dissociated suspensions of CNS from unmanipulated pathogen-free mice contained appreciable numbers of T cells, also of a naive phenotype. Their study went on to show that if the naive T cells recognized myelin antigens, then they were inactivated in the CNS through a process of anergy, unless a costimulatory stimulus was also present (51).

Taken together, the two studies suggest that the CNS is routinely 'patrolled' by T lymphocytes, and that the outcome of their recognition of antigen is determined by co-stimulatory signals e.g., by the local milieu. In cases where there is no possibility of antigen recognition (e.g., OVA), activation potential is maintained. This interpretation satisfies a number of immunological tenets, including that of immune surveillance, and provides a mechanism whereby the process of epitope spreading in the CNS (see below) may draw on a naive pool of T cells to expand the repertoire during anti-viral or autoimmune responses (1, 53). It should be kept in mind that neither study localized naive T cells within the CNS, and the isolation procedures used may bias towards cells in perivascular compartments. Nevertheless, this is exactly where the majority of T cells are found in EAE and MS (54, 55), so the possibility that naive T cells localize to the same site supports the general relevance of the findings.

4.2. Antigen presentation and response induction in CNS

MHC II and costimulator ligand-expressing cells are critical for induction of CD4-positive T cell immune responses (56). Immune dogma holds that such 'professional' antigen-presenting cells are restricted to lymphoid tissue. This is largely supported by studies such as those of Jenkins and colleagues (57). Nevertheless, the fact that naive T cells can be found in non-lymphoid tissue inevitably raises questions whether their induction in those tissues might be possible. The phenomenon of Epitope Spreading during autoimmune and antiviral responses in the CNS adds weight to these questions (1, 53).

4.2.1. Epitope Spreading

Epitope Spreading is exemplified by the emergence of proteolipid protein (PLP)-specific T cells in mice that were infected with TMEV (58), but has been shown in other systems, including MS (59). It is assumed that the induction of costimulator ligands during viral infection permitted the presentation of endogenous myelin antigens, perhaps phagocytosed consequent to virally-induced damage. This raises the question whether naive PLP-specific CD4-positive T cells became activated within the CNS. The studies of naive T cell entry to the CNS (above) demonstrate the potential availability of such T cells. The question then becomes whether there is antigen-presenting capacity within the CNS sufficient for their activation. Two cell types compete for attention in this regard, macrophages and microglia.

4.2.2. Macrophages

Macrophages have been shown to be essential for induction of EAE in rats and mice. Depletion of peripheral macrophages using liposomes, silica or other techniques blocked disease onset (reviewed in ref. 20, also see ref. 60). Blockade was effective even when EAE was adoptively transferred to controls with already-activated CD4-positive T cells, arguing against an effect at the level of T cell induction. In one study, leukocytes accumulated in the perivascular space of macrophage-depleted mice, and levels of activation-associated mRNA's were only marginally affected (60). The inference was that the role of macrophages in EAE includes facilitation of immune cell entry to the CNS. Subsequent studies showed that leukocyte accumulation did not occur if the adoptively-transferred T cells were specific for an irrelevant antigen (OVA; ref. 20). This pointed to an antigen-presenting role for macrophages, and indirectly suggested that the perivascular macrophage may play an important role through presentation of antigen to potentially encephalitogenic T cells. Perivascular cells have also been shown to be critical for induction of anti-Simian Virus responses in CNS (61). It can then be hypothesized that antigen presentation in perivascular space by so-called gatekeeper macrophages facilitates the entry of encephalitogenic T cells (20, 31). The fact that non-myelin-specific T cells which have been described in the CNS were of a naive phenotype is consistent with this hypothesis, because such cells would not recognize antigen on the Gatekeeper cells. Furthermore, the fact that regions of the brain where blood/CSF exchange is relatively unrestricted are not usually infiltrated in EAE or other inflammatory conditions may reflect lack of essential MHC II-positive antigen-presenting gatekeeper cells at those sites (29). The nature of the change imparted to T cells by this antigen presentation has not been defined, but may include upregulation of chemokine receptors. Also, it is not clear what is the mechanism whereby naive non myelin-specific T cells can cross the BBB.

4.2.3. Microglia

Microglia derive from bone marrow and share expression of the CD45 or LCA molecule with other bone marrow-derived cells. They colonize the CNS in neonatal animals and become isolated from other blood cells when BBB integrity is established early in life (32). There are no phenotypic markers that can distinguish microglia from macrophages, especially when both are activated. In rodents, a subpopulation of cells from CNS isolates that express slightly reduced levels of CD45 have been identified as microglia, and can be fractionated by cell-sorting (35, 62, 63). Another approach that has been used is to reconstitute lethally-irradiated animals with phenotypically-distinct bone marrow. Because CNS microglia are not replaced in the time course of most experiments, they can then be distinguished on this basis (32). Fractionation on the basis of these phenotypes has allowed comparison between macrophages and microglia from virus-infected and autoimmune CNS (63).

Microglia are the only cells that express MHC II in the uninflamed CNS (32, 33). They have been assumed to present antigen to CD4-positive T cells. In vitro analyses confirm this capability, and show that microglia can induce a primary T cell response, as well as secondary T cell activation (35, 64, 65). Astrocytes, by comparison, can only promote secondary responses, and only after induction of MHC II, e.g., by IFN-gamma (66, 67). Microglia induce T cell proliferation and Th1 cytokine production, the latter consistent with the ability of microglia to produce IL-12, and to express B7.1 and B7.2 (66). Microglial expression of co-stimulatory molecules is critical for CNS immunopathology (68-70).

However, microglia differ from macrophages in the extent of T cell response that they can induce, and in the outcome of these responses. T cells that are stimulated by microglial antigen proliferate less than those stimulated by macrophages, and they progress more readily to death by apoptosis (34). Whether these *in vitro* findings reflect real *in vivo* differences is unclear, but they do offer a mechanism for the relative immune privilege of the mature CNS. It is likely that infiltrating macrophages play a major role as antigen presenting cells in the CNS in EAE, but microglia may be critical for initiation of responses before infiltration is widespread.

Both microglia and macrophages can phagocytose tissue and cellular debris, including myelin, and they likely play a major role in this critical activity in inflamed CNS. Activated, myelin debris-containing microglia/macrophages have been identified by histological and magnetic resonance imaging methods, coincident with MS plaques, and by autoradiography and histology in EAE lesions (71). The myelin-phagocytic activity of microglia/macrophages has led to their being proposed as critical mediators of demyelination, and they are prominent in many MS plaques (72).

5. IMMUNE EFFECTOR FUNCTION IN CNS

Infiltrating CD4-positive T cells exert their effect in CNS primarily via the production of cytokines. The guiding concept is that of cytokine cascades, initiated by antigen-presentation as T cells enter the CNS, then progressively involving glial cells and other infiltrating cells, with feed-forward and feed-back loops for their

regulation (73). The cytokine cascades and cellular interactions that have been described are already almost too numerous to review, and addition of those that may reasonably be proposed would generate an enormously complex network. I will focus on two groups of mediators that illustrate the principles and complexity underlying CNS immune responses.

5.1. IFN-gamma

As already discussed, the Th1 cytokine profile, characterized by production of Type II or immune interferon, IFN-gamma, is required for induction of EAE, and is associated with anti-viral immune responses. The Th1 cytokine IFN-gamma and mediators induced by it are detected in CNS tissue and infiltrating cells, as well as in cerebrospinal fluid (CSF) and blood cells, in MS and EAE (73). The IFN-gamma induces expression of cytokines, adhesion, MHC and co-stimulatory molecules (73, 74). Correlation between kinetics and level of expression with disease progression and severity have suggested a causative role (Guilt by Association) for IFN-gamma (20, 73). The IFN-gamma is the only cytokine associated with immune responses in the CNS that is not itself produced by CNS-resident cells, and so is an important diagnostic for CNS immunity. Effector functions of IFN-gamma include induction of cytokine (especially TNF) and chemokine production by other infiltrating cells and by glial cells. Transgenic expression of IFN-gamma in CNS has generated demyelinating phenotypes, and also more subtle phenotypes that qualify as pro-inflammatory (elevated MHC I, iNOS; ref. 73). In rats, IFN-gamma induces inflammation when injected into the CNS (75, 76). Administration of IFN-gamma in an MS clinical trial increased relapse rate and severity (77). These briefly-summarised findings support a pathologic role for IFN-gamma . By contrast, similar to Type I IFN's, that are also anti-virals, IFN-gamma suppresses lymphocyte proliferation. This is considered one reason for confusing results from inhibitor studies, which consistently exacerbated rather than blocked EAE.

In the absence of IFN-gamma, the profile of chemokines produced in EAE is radically shifted, with a corresponding shift from macrophage to neutrophil infiltration, and loss of perivascular association of the infiltrating cells. The chemokines RANTES, MCP-1 and MIP-1alpha, become almost undetectable in IFN-gamma deficient mice with EAE, and MIP-2 and TCA-3, previously undetectable, become prominent (48). It is important to qualify this observation with a parallel finding, that in an immune- and IFN-gamma-independent response to axonal lesioning, glial cells in hippocampus produce all of the above chemokines (Babcock and Owens, unpublished). Their expression is not therefore IFN-gamma-dependent. However, IFN-gamma is necessary in conjunction with the signaling event(s) that induce chemokines in EAE. The role of chemokines in CNS immunity and repair responses was recently reviewed by us and will not be dealt with further here (http://www.eurekah.com/reports/immunology/santamaria/07/babock7.html).

5.2. Reactive oxygen and nitrogen intermediates

Studies of immune responses against parasites established that both nitric oxide and reactive oxygen species (ROS) such as superoxide were effective cytopathic agents, conferring host protection. This prompted examination of their role in CNS inflammation.

5.2.1. Nitric oxide

Nitric oxide is formed by nitric oxide synthases (NOS), a family of enzymes encoded by different genes. Inducible nitric oxide synthase (iNOS), or NOS2, the enzyme responsible for rapid, high level, calcium-independent NO production, is expressed by inflammatory leukocytes such as macrophages and neutrophils (78). Its expression is transcriptionally regulated, one of the primary inducing agents being IFN-gamma. Findings of elevated gene and product expression in MS and EAE were interpreted to show a role for these reactive intermediates in demyelination and oligodendrocyte damage. Initial inhibitor studies in rats, which blocked EAE, supported this interpretation. However, inhibitor studies in mice gave various results, in many cases causing exacerbation of EAE (reviewed in ref. 79). Mice that lacked a functional iNOS gene showed exacerbated EAE (more severe symptoms) compared to wild-type controls (78, 80). NO has been shown to suppress T cell proliferation (81), and studies from Willenborg and colleagues have suggested it to be the mediator responsible for the anti-proliferative effect of IFN-gamma (82).

5.2.2. Superoxide and peroxynitrite

Nitric oxide combines with superoxide (O_2^-) to form the strongly oxidative and cytopathic mediator peroxynitrite ($ONOO^-$). Superoxide is produced via action of NADPH oxidase, or phagocyte oxidase (phox). Deficiency in expression of this enzyme causes chronic granulomatous disease in humans, and gene-knockout mice show analogous defects, when not maintained under pathogen-free conditions (83). Peroxynitrite is produced by microglia and astrocytes in EAE (84, 85), and both monocytes and astrocytes produce $ONOO^-$ in MS (86). Mice deficient in NADPH oxidase were resistant to EAE (87). It has been suggested that the rapid reaction by which superoxide (O_2^-) combines with NO to form peroxynitrite ($ONOO^-$) depletes NO, and that this is a significant factor in controlling the immunoregulatory effect of NO (87). However, peroxynitrite has been shown to induce T cell apoptosis (88), $ONOO^-$-producing cells in EAE were localised in proximity to apoptosing (TUNEL-positive) inflammatory cells (89), and $ONOO^-$ was invoked as one mechanism for T cell suppression by granulocytes *in vitro* (90). Interplay between iNOS-derived NO and superoxide may determine whether a cell at an inflammatory lesion is pro- or counter-inflammatory.

5.3. Immunoregulation by cytokines

Immune responses are controlled by a variety of mechanisms, ranging from clearance of inducing antigen to induction of complex immunoregulatory networks. Th1 versus Th2 inter-regulation is perhaps the most commonly

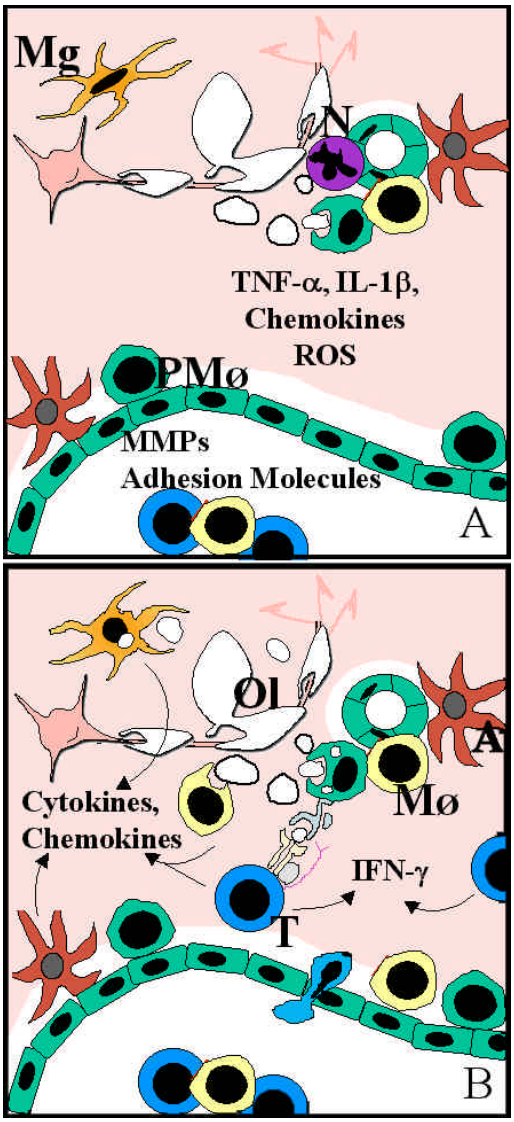

Figure 1. Innate vs. T-cell mediated events in CNS immune response. Panel A illustrates an innate program of reactivity to demyelination within the CNS. The BBB limits cell entry, and "gatekeeper" macrophages that patrol the CNS phagocytose myelin debris. Expression of proinflammatory cytokines, chemokines, adhesion molecules and MMPs allow the first cascade of immune cells to the site of injury: macrophages and neutrophils. Reactive oxygen species are produced, and signals for specific immunity are initiated. The CNS immune response has progressed in Panel B. Myelin-reactive T cells infiltrate the CNS, secreting IFN-gamma, which modifies and amplifies the production of inflammatory mediators by a variety of cell sources. Upregulation of MHC and costimulatory molecules allows for efficient antigen presentation by macrophages and activated microglia.Abbreviations used: PMØ, Perivascular macrophage; MØ, macrophage; Mg, microglia; O, Oligodendrocyte; A, Astrocyte; N, Neutrophil; T, Tcell; ROS, Reactive oxygen species; MMP, Matrix metalloproteinases.

invoked and frequently studied 'network' in recent years. Th2 CD4-positive T cells, characterised by production of IL-4 and IL-5, inhibit the differentiation and activity of Th1 CD4-positive T cells (17, 18). Some studies have shown temporal correlation between expressions of Th2 cytokines in CNS with remission from EAE (91). The cytokine Transforming Growth Factor-Beta (TGF-beta) is also immunosuppressive and has been implicated in immunoregulation in CNS (92). There has been much interest in exploiting cytokine 'switching' for immune therapy of CNS inflammatory disease, one instance being the use of altered peptide ligand (APL) therapy. The APL's are MHC-binding peptides with substitution of one or a few amino acid residues, such that TCR signaling is modified (93). This can alter the cytokine profile of peptide-specific T cells. Use of APL's corresponding to encephalitogenic myelin proteins has alleviated experimental disease, and has been shown to switch MS CD4-posistive T cells from a Th1 to a Th2 phenotype (73, 93).

One potential problem with cytokine switching as a means of effecting immunoregulation is the complexity of cytokine function. Thus, Th2 cytokines not only inhibit Th1 responses, but they also induce humoral responses, which themselves exert effector function. This was invoked as the reason for increased severity of EAE in marmosets after they had been treated with a myelin APL (94). Similarly, TGF-beta promotes glial activation, which may be beneficial for AD (95), but can also induce more detrimental pathology (96). Consideration may need to be given to cell source and timing of cytokine immunoregulation.

Whether cytokine immunoregulation plays a role in normal CNS functioning, and whether CNS sources of potentially immunoregulatory cytokines are important, are questions to which are no clear answers as yet. Innate immunoregulation in CNS may include action of TNF, IL-10, and IL-18 (73). The expression of all of these (and other) cytokines by glial cells is influenced by immune mediators, especially by IFN-gamma in different ways. Innate programs of production and effect of TNF were not significantly altered by IFN-gamma in models of CNS injury (97), whereas IL-10 production in CNS was absolutely dependent on IFN-gamma (48).

5.4. Protective/repair functions of T cells and microglia

Finally, it must be considered whether infiltrating immune cells play any role beyond that of clearing infectious pathogens from CNS. There is a need for regeneration and repair following the tissue damage that accompanies infection, the inflammatory response that ensues, and/or the autoimmune attack that occurs in MS or EAE. A curious paradox is emerging whereby the same T cells and macrophages which infiltrate to initiate inflammation and even autoimmunity are themselves sources of protective and regenerative factors. Thus, T cells that infiltrate the CNS in MS produce neurotrophins (98). Similarly, activated microglia and astrocytes are sources of protective neurotrophins in CNS (99). Infiltrating MBP-specific CD4-positive T cells accelerated regeneration of injured optic nerve or spinal cord in rats and mice, and

these effects were not mimicked by OVA-specific, CD4-positive T cells (100). Furthermore, deliberate immunization of mice against myelin proteins promoted accelerated recovery from CNS injury (101). Analogously, infiltration of macrophages to sites of nerve injury appears to be a limiting factor in promoting recovery (102). Absence of CNS regeneration was overcome when macrophages had been activated with peripheral nerve segments (peripheral nerve has a much greater capability for re-growth; ref. 103). Thus, myelin-specific T cells and activated macrophages are both critical for CNS repair, in promoting protection of existing neurons and axonal regeneration, respectively (104).

It seems that the immune system is not only designed for access to the CNS for purposes of host-protection against infections, but may also have evolved a role as a counterpart to its wound-healing role in the vasculature and peripheral tissues. CNS immunoreactivity invokes both innate and T-cell mediated responses, predisposing for host-protective, regenerative as well as pathologic outcomes (Figure 1). Immune responses in the CNS, therefore, are an integral component of normal function of both the nervous and immune systems.

6. ACKNOWLEDGEMENTS

Trevor Owens wishes to thank the members of his laboratory for their continued inspiration.

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Key Words: Immunology, Brain, Spinal Cord, Microglia, T Lymphocytes, Cytokines, Chemokines, Review

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