Recent Observations on the Experimental Pathogenesis of Multiple Sclerosis

Scott Sloka, Ph.D. (Memorial 2003)

Abstract
Multiple sclerosis is a disease with complex immune system interactions that are being painstakingly elucidated by various means of experimentation. Great effort is being put forth to discover disease processes that may be affected by immunomodulatory therapies. Difficulties of experimentation and the ability to compare separate experiments in the same context notwithstanding, results from various experiments are being collated and many logical disease mechanisms are being proposed. This paper is an overview of new experimental observations made in the context of previous science and presents a perspective on the ability to compare experimental results from various contexts.

Introduction
Multiple sclerosis (MS) is a chronic, demyelinating disease of the central nervous system white matter that may cause paralysis, sensory disturbances, incoordination, visual impairment, and alterations in bowel and bladder function. The precise etiology of MS has not been elucidated; however, many observations have been made in both human MS patients and in animal models of the disease, and these observations suggest that both genetic susceptibility and environmental factors play a role in the etiology of MS. Some features of the disease suggest that it shares characteristics of other autoimmune disorders whereby dysfunction of the local immune system is associated with a local inflammatory reaction. The pathological lesion of MS involves demyelination of central nervous system (CNS) axons and/or injury to either oligodendrocytes or axons, associated with inflammation that surrounds venules of the CNS and extends into the myelin sheath. This demyelination and associated axonal injury causes the clinical features of MS.

The intensive and progressive experimental study of MS has elucidated many key observations in the pathogenesis of the disease. This paper is a review of both recent and historical observations of the pathogenesis of MS and it attempts to organize these observations in a manner that suggests a temporal sequence of causative events. However, some caution must be given with respect to the hypothesized sequencing since most of the observations have been made in a controlled environment, and the sequencing itself has not been the focus of these studies. A brief explanation of the methodology used in the collation of these results is given to lend context to the inclusion of some observations and not others.

Methodology
This compendium of observations was derived from several sources. Historical observations are taken from several excellent review papers, and recent observations were taken from a literature search of the period from Oct 2000 to Oct 2001 using MEDLINE. 171 new results were found, and of these, only those that contribute to modes of entering of activated T cells into the CNS, modes of T cell activation, and modes of possible oligodendrocyte and axonal injury are included. Other observations such as the considerable advances in our understanding of the involvement of B cells and antibodies in the MS lesion or the contribution of different myelin component epitopes to autoimmunity were not included. As well, when some observations lacked a sequential step, an historical search was made to find the missing observation to complete the sequence. It was not always possible to find the missing steps, providing evidence for the active research in this area.

Modes of Entry
MS is believed to be an inflammatory attack on the central nervous system involving the immune system, and is associated with dysregulation of the immune system, possibly including multiple modes of dysfunction. Generally, the CNS is protected from the systemic immune system by the blood-brain barrier. However, entry of immune cells into the CNS is possible and this entry likely contributes to the inflammatory reactions noted in MS. Activated immune cells do gain entry, and this section describes mechanisms whereby T cells may enter the CNS. (Figure 1)
Figure 1. This diagram depicts four steps that may contribute to the pathogenesis of MS. T cells may be immunologically activated anywhere within the body and may travel throughout the bloodstream releasing IFN-γ, IL-2 and TNF-α. IFN-γ and TNF-α induce the expression of ICAM-1 and VCAM-1 on cerebral vascular endothelium. α4 integrin binds to VCAM-1 molecules, CD4 binds to MHC class II molecules and LFA-1 binds to ICAM-1. Activated, bound T cells may extravasate and secrete Gelatinase A and B, which is capable of breaking down basement membrane. T cells that enter the brain may then activate the macrophage system via IFN-γ. Whether all these events are chronologically related is not known.
Capillary endothelial cells in the CNS are nonfenestrated, are connected with tight junctions, and rest on an extracellular matrix (ECM) of type IV collagen. The cell adhesion molecules, ICAM-1, VCAM-1 and E-selectin, are not normally expressed on the vascular endothelium. It has been observed that activated T cells that are reactive to a component of myelin, called myelin basic protein (MBP), secrete IFN-γ, TNFα and IL-2 (a T cell stimulatory cytokine). These activated T cells express α4 integrin that binds to VCAM-1 molecules, CD4 that binds to MHC class II molecules and LFA-1 that binds to ICAM-1. Therefore, activated T cells are capable of inducing expression of adhesion molecules on CNS endothelial cells, thereby creating a means of extravasation, at least to the basement membrane. It has been observed that any activated T cell (not only T cells specific to myelin products) can extravasate, and regularly do.

Interestingly, other mechanisms may permit the expression of adhesion molecules – viral infection and metabolic stress both upregulate expression of ICAM-1, VCAM-1 and E-selectin. As well, differential expression of adhesion molecules occurs in the various clinical forms of MS. Intrathecal synthesis of sVCAM-1 is present in the relapsing-remitting and secondary progressive forms of MS, intrathecal synthesis of sICAM-1 has been observed in all clinical forms of MS, and MS patients with progressive forms of the disease were characterised by intrathecal synthesis of sL-selectin. These observations suggest that the pathogenesis of MS may be modulated by external factors and may contribute to clinical heterogeneity.

Once the T cells extravasate, the basement membrane barrier of type IV collagen prevents further entry into the CNS. However, activated T cells and natural killer (NK) cells can secrete gelatinase A and B. These enzymes are detectable in the spinal fluid of patients with MS and other inflammatory neurological diseases, and gelatinase B immunoreactivity is seen in MS lesions. These matrix metalloproteases (MMP) have specificity to collagen type IV and are thus able to degrade the basement membrane. In fact, the prevention of T cell entry is currently used as immunomodulatory therapy using IFN-β1a and IFN-β1b (Avonex and Betaseron, respectively), IFN-β being a potent inhibitor of both gelatinase A and B. Therefore, it is possible for activated T cells to pass through both barriers of the CNS. As well, gelatinase A and B can induce the release of TNFα from a cell-bound form to a soluble form, and TNFα induces vascular leakage (and activates monocytes and neutrophils, cells observed in CNS inflammation). Thus, the entry of activated T cells can also be self-sustaining.

In terms of the time course of the above events, it is difficult to determine, experimentally, whether one observation follows another. As well, in vitro observations do not necessarily follow in vivo observations. However, recent work has noted the time course of some of the events in the entry of T cells. The transcription of TNF-α/β and IL-2, known modulators of MMPs, was noted to be upregulated only in distinct stages of lesion formation. As well, their receptors were not induced at all experimentally, which suggests that additional signaling molecules participate in the sustained upregulation of gelatinase A and B in multiple sclerosis.

**Modes of Activation**

T cells are observed in active MS lesions and are known to participate in the inflammatory reaction. Most activated T cells are differentiated towards either a Th1 or a Th2 population, and each are distinguished by the types of cytokines they release. Th1 cells are active in the initiation of inflammation and secrete IL-12, TNF-α and IFN-γ. Th2 cells may activate B cells through IL-4,5,6,10,13, and may cause antibody mediated injury and antibody mediated remyelination. Activation and subsequent differentiation of T cells requires an antigen-specific recognition of CD4, TCR and MHC class II receptors as well as an interaction between B7-1/B7-2 and CD28/CTLA-4 costimulatory receptors.

B7-1, B7-2 and CD28 are differentially expressed throughout the course of disease. B7-1 (CD80) and B7-2 (CD86) receptors on antigen presenting cells (APCs) decrease the TCR activation threshold (may enhance lower avidity reactions) in T cells when bound to CD28 on T cells, and can activate an immune response or differentiate T cells to a Th1 subset. CD28+TCR-mediated signals results in secretion of cytokines, upregulation of CTLA-4 mRNA, and T cell proliferation and differentiation. CTLA-4 is expressed by activated T cells. B7-1 and B7-2 receptors on APCs increase the TCR activation threshold in T cells when bound to CTLA-4 on T cells leading to either anergy or Th2 differentiation, depending on the activation status. CTLA-4 delivers a downregulatory signal to activated T cells and may also mediate the induction of peripheral tolerance.

Why does the activation via CD28/CTLA-4 matter for the study of MS? A large body of knowledge has been gained through mice models of MS and it has been shown that manipulation of the B7-CD28/CTLA-4 costimulatory pathways (via CTLA-4Ig) can prevent the initiation of experimental autoimmune encephalomyelitis (EAE) a mouse model of MS. This appears to be a fruitful avenue to explore as a treatment of MS, and further work is moving towards that direction. There are differences in proportions of B7 subsets in active lesions, with increased expression of B7-1 being noted on lymphocytes in active periventricular lesions and increased expression of B7-2 being noted on macrophages.
Modes of Injury

Once the inflammatory reaction has been initiated, several mechanisms of injury are possible. They may include, but are not limited to: cytokine-mediated injury of oligodendrocytes and myelin, phagocytosis of myelin by macrophages, complement-mediated injury of oligodendrocytes and myelin, and direct injury of oligodendrocytes and myelin by CD4+ and CD8+ cells. This section is an overview of these possible mechanisms, concentrating on the mechanisms that are specific to inflammation in the CNS.

Nitric Oxide

Local inflammatory molecules may injure oligodendrocytes. One such cytotoxic molecule is nitric oxide (NO). NO is involved in the killing of oligodendrocytes by microglial cells, and both IFN-γ and TNFα can induce induced nitric oxide synthase (iNOS) in astrocytes, microglia and macrophages. IFN-γ and TNFα are inflammatory cytokines elicited by the Th1 subset of T cells, described above, and iNOS is an induced enzyme that produces NO. Expression of iNOS has been found in MS lesions. As well, both NO and TNFα damage myelin and induce phagocytosis of myelin by macrophages. Therefore, inflammatory states may induce oligodendrocyte destruction by NO.

T Cell-Mediated Injury of Oligodendrocytes

Direct cell-to-cell injury is also possible in the CNS. Activated MHC-restricted CD8+ T cells may become cytotoxic to oligodendrocytes. Activated CD4+ and CD8+ αβ T cells are both present in MS lesions. For CD8+ cells to be cytotoxic to other cells, MHC-restricted recognition of a peptide-MHC class I complex by the T cell receptor (TCR) is necessary. MHC class I and II are not generally expressed on oligodendrocytes. However, in-situ expression of MHC class II receptors in endothelial cells, microglial cells, and oligodendrocytes (but not astrocytes) has been described in the presence of the overexpression of IFN-γ in the CNS. Also, after incubation with either IFN-γ or TNFα, oligodendrocytes may acquire MHC class I expression. Additionally, it has been shown that human oligodendrocytes expressing MHC class I molecules are susceptible to alloreactive class I-directed CD8+ cytotoxic T cells. Further evidence for the possibility of this mechanism lies with an experiment where-by CD8+ T cell lines reactive to MBP peptide in an HLA-A2 groove were cytotoxic to HLA-A2 oligodendrocytes but not to non-HLA-A2 oligodendrocytes, and was inhibited by anti-MHC class I antibodies. However, this mechanism is not necessary to cause all the demyelination in the CNS because, experimentally, demyelination still occurs in CD8+ depleted mice, with marked reduction in neurological impairment.

Natural Killer Cell-Mediated Injury of Cells in the CNS

The contribution of natural killer cells (NK) towards cell injury in MS has been studied. CD16/CD56+ cells (NK cells) have been found in MS plaques. CD56 is a member of the neural cell adhesion molecule family and is expressed on NK cells, some cells of the central nervous system, and on some subpopulations of CD8+ and CD4+ T cells. CD56 expression and cytotoxicity is dependent on the activation state of the effector cells (T cells, NK cells), and may depend on the presence of myelin-restricted T cells or antibody. Human MBP-reactive CD4+ T cells that express CD56 have been shown to be cytotoxic to some target cell populations in the CNS in a non-MHC-restricted fashion. This area of research is ongoing, possible temporal sequencing for this mechanism, as well as the requirements of the local environment, are still being studied.

Antibody-Mediated Oligodendrocyte Cytotoxicity

There has been much controversy surrounding the role of antibodies in the MS lesion. Antibodies auto-reactive to CNS components have been measured in the serum and CSF of MS patients and they are autoreactive to: myelin components, oligodendrocyte proteins, cell nuclei, endothelial cells, fatty acids, gangliosides, and axolemma. The role of these autoantibodies is uncertain; however, recent studies have indicated the possibility of antibody-mediated oligodendrocyte cytotoxicity. Evidence for the pathogenesis of antibody-mediated injury of oligodendrocytes is demonstrated by the presence of complement in MS lesions, the fact that myelin is a complement activator (via the classical pathway), and observations that myelin may lead to lytic injury of cells. Antibodies can be cytotoxic by activating complement. However, the addition of myelin-specific antibodies can also enhance the degree of demyelination in T cell-mediated inflammation, markedly augmenting the extent of tissue injury through antibody-dependent cell-mediated cytotoxicity (ADCC). The presence of myelin antibodies attached to the surface of myelin may trigger macrophage attachment and subsequent phagocytosis. Antibody Fc receptors are upregulated on macrophages, microglia and NK cells in MS lesions and may be of functional importance in ADCC, phagocytosis, and local immunoregulation. Research towards understanding the role of antibodies in the MS lesion is ongoing, but there is no consensus on their actual contribution at the present time.

Tumor Necrosis Factor as a Mediator for Oligodendrocyte Damage

Tumor necrosis factor alpha (TNFα) is thought to be one of the mediators responsible for the damage of oligodendrocytes in multiple sclerosis (MS). TNFα is produced by both macrophages and microglia in MS lesions and precedes the production of IL-12. IL-12 promotes the acquisition of a Th1 cytokine profile by CD4+ T cells, which also secrete TNFα. Activated MBP-reactive T cells secrete IFN-γ, TNFα and IL-2. As well, levels of TNFα correlate with clinical disease progression. Administration of TNFα to primary cultures of oligodendro-


cytos induces DNA fragmentation and significantly decreases the number of live oligodendrocytes in vitro. High dose TNF-α induces apoptosis in human oligodendrocytes and caspase-1-mediated cell-death pathway is activated in TNF-induced oligodendrocyte cell death. A pathway that may be relevant in TNF-α-mediated cytotoxicity is thought to be the direct ligand binding of TNF-α to an apoptotic receptor, TNF-R1. The TNF-R1 (TNF-R55) receptor is responsible for the induction of apoptosis (TNF-mediated cytotoxicity) in most human cases. Members of the TNF-R family are typical signal sensors which, upon binding with ligand, aggregate and recruit signal transducers with TNF-R1 activating the apoptosis cascade. TNF-R1 has been found on oligodendrocytes around MS lesions. Curiously, these surrounding cells were not found to undergo apoptosis, thus suggesting some unknown mechanism of apoptotic resistance that differentiates these cells from cells within MS lesions that do undergo apoptosis.

The decrease in symptoms of women with MS during pregnancy may, at least partly, be explained by the effects of TNF-α on the inhibition of the production of iNOS. Estrogens, progesterone and estriol (at concentrations consistent with late pregnancy) have been found to inhibit both TNF-α and NO production by microglial cells, thus potentially reducing the inflammatory state of the disease.

**Apoptosis and Oligodendrocytes**

CD95 (Fas) is a member of the TNF receptor family, and the engagement of this receptor with a CD95 ligand (FasL) causes activation of the caspase cascade, leading to cell death. Fas is also involved in modulating the immune response. Experiments have demonstrated a difference in the involvement of the Fas-mediated apoptosis pathway in MS patients with different clinical presentations. Fas-induced cell death was significantly lower in patients with secondary progressive MS (SPMS) than in patients with relapsing remitting MS (RRMS). Therefore, this pathway may play a role in the pathogenesis of MS.

CD95 is not expressed in normal, adult, human brain tissue, although it has been found on fetal human astrocytes. However, CD95 has been found on oligodendrocytes in MS lesions. CD95L-bearing cells in MS include macrophages, microglia and lymphocytes. Expression of the CD95 receptor is increased in IFN-γ treated human oligodendrocytes and they become susceptible to CD95-mediated apoptosis. IFN-γ, however, is not directly cytotoxic. IFN-γ and TNF-α possibly upregulate the expression of CD95 on a number of neural cell types, not only oligodendrocytes. Therefore, the possibility exists that activated T cells in the brain parenchyma may cause the upregulation of CD95 receptors through IFN-γ, giving CD95L-bearing cells the chance to initiate the apoptosis cascade, causing cell death. The observation that both the expression of FasL was increased in patients with MS compared with healthy control subjects in unstimulated peripheral blood mononuclear cells, and TNF-related apoptosis-inducing ligand (TRAIL) receptor 2 (TRAIL-R2) mRNA levels were also upregulated in patients with MS, lends weight to the suggestion that this is a relevant mechanism in the pathogenesis of MS. Interestingly, a higher expression of CD95 on NK cells has been observed in the remission of MS.

**Axonal Injury**

Loss of brain parenchymal volume in patients with MS had been previously assumed to be predominantly confined to white matter. However, by monitoring levels of N-acetylaspartate (a putative marker of axonal integrity), proton MR spectroscopy has recently demonstrated that the extent of axonal injury associated with white matter inflammation and demyelination is significant and had not been well appreciated from classical pathology studies. Particularly, morphometric analyses have shown that up to 50-80% of axons may be lost in chronically demyelinated cervical spine plaques. In fact, magnetic resonance spectroscopic imaging measurements of brain N-acetylaspartate show that this axonal injury may occur even in the absence of clinically evident functional impairments. It has also been demonstrated that axonal injury occurs even in the earliest stages of multiple sclerosis. Interestingly, acute axonal damage, as defined by the accumulation of amyloid precursor protein (APP), has been correlated with the number of macrophages and CD8-positive T lymphocytes within the lesions but not with TNF-α or nitric oxide synthase expression. This suggests that axonal injury may not be solely due to demyelination. However, the mechanisms of axonal injury are largely unknown at this time. In particular, it is not clear whether inflammatory effects may damage axons directly or whether they operate primarily through a pathway that includes demyelination.

**Discussion**

The pathogenesis of MS is carefully being elucidated through progressive experimentation involving both human and animal models of disease. Mechanisms for the activation and entry of T cells into the CNS, and mechanisms for the inflammatory destruction of brain parenchyma, have been proposed. Some recent observations were presented above in the context of both previous science and already-established proposed mechanisms with the caveat that the temporal sequence of all these observations has not completely been resolved. In the case of separately performed experiments, it is difficult to draw conclusions about the temporal sequencing of events that are not in the complete context of the in vivo environment. This temporal sequence is especially important when attempting to construct the initiating events of the disease, and it is the hope that the future knowledge of these events will lead researchers to means by which the disease process might be controlled.
MS is studied using several different laboratory and diagnostic methods including: the use of animal models of disease; the observation of CNS specimens from patients with and without MS; the in vitro culture of specimens from either animal models or patients with and without MS; and the use of imaging technology, including magnetic resonance imaging (MRI). Each of these methodologies has its own advantages and disadvantages that affect the strength of conclusions that can be drawn from experimental results, and therefore an understanding of the source of these observations and the potential problems with each methodology is important for the understanding of the context of these observations.

Experimental autoimmune encephalomyelitis (EAE) is an animal model that has been used to study MS; EAE has been induced in many species (monkeys, sheep, dogs, marmosets, chickens, rats and mice) although rodent models are preferentially used. There are three major clinical forms of EAE: hyperacute with rapid onset and death; acute with transient paralysis and subsequent recovery or steady state; and a chronic relapsing-remitting type. EAE is induced in rodents by the administration of spinal cord or myelin components from cows, guinea pigs, rats, or mice. Commonly, myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG) are used to induce EAE (with complete Freund's adjuvant), and the administration of B. pertussis improves the induction of disease. The MHC-restriction and TCR restriction to both MBP and PLP epitopes is an important factor when considering the evidence derived from murine models – the induction of EAE in mice will vary according to the genetic makeup of the MHC and TCR genes, and therefore observations may be different depending on the strain of mouse. The variable effects of active immunization may be avoided by using the adoptive transfer of autoreactive CD4+ T lymphocytes to induce EAE. Ostensibly, the ability to draw conclusions from EAE experiments towards our understanding of MS is at least partly affected by both the variation between species (human and rodent), and the artificial induction of a disease state that may or may not be representative of the pathophysiological context of MS in humans. Events may be initiated out of sequence (causing artificial elevations of factors under observation) and species-specific differences in local microenvironments may produce immune responses that are also species-specific and not generalizable.

MRI has been used to study the progression of MS. Possible disruption of the blood-brain barrier and inflammatory changes in MS lesions are events in the natural progression of MS that may be measured with T2 weighted MRI or postcontrast Gadolinium T1 weighted MRI. Correlation to pathology is imprecise, and increased signal on T2-weighted images lack pathological specificity as they can reflect edema, inflammation, or longstanding disease characterized by tissue destruction. Therefore, there may be some difficulty precisely diagnosing acute lesions on T2 MRI. Hypointensities on T1 weighted enhanced MRI may be associated with acute lesions. As well, variations in the dosage of contrast also alter the number of enhancing lesions. New quantitative MRI techniques, such as cell-specific imaging, magnetization transfer imaging (MTI), proton magnetic resonance spectroscopy (MRS), diffusion-weighted imaging (DWI) and functional MR imaging (fMRI) have all been recently applied to the study of MS. These techniques should provide more accurate and pathologically specific estimates of the MS lesion burden and may non-invasively provide clues to the temporal sequence of events during the natural history of the disease, which is key to our understanding of the disease pathogenesis.

Given that many excellent observations have been made in various contexts, it is difficult to ascertain whether all of these observations are possible in the context of one human individual with MS, especially given the heterogeneity of the disease. Interspecies variability most definitely influences the inclusion of proposed mechanisms involving observations from mixed species. Intraspecies genetic variability also plays a role, especially in terms of genetic susceptibility. And yet, even though some of these considerations cloud our certainty, additional disease mechanisms proposed by others in the context of our knowledge of other autoimmune disease have added to the benefits gained through experimentation. Certainly, future experimental observations will resolve both the interspecies and intraspecies variability issues and will elucidate a temporal sequence that will aid in the discovery of treatments of MS. It may be that we do not need a complete sequence of events, temporally ordered, unique to our species, to find better means to treat MS, and that only a subset of observations will provide sufficient insight into the natural history of the disease. Hopefully, this is indeed the case.

References


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