Pathogenic CD8 T cells in multiple sclerosis and its experimental models

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A growing body of evidence suggests that autoreactive CD8 T cells contribute to the disease process in multiple sclerosis (MS). Lymphocytes in MS plaques are biased toward the CD8 lineage, and MS patients harbor CD8 T cells specific for multiple central nervous system (CNS) antigens. Currently, there are relatively few experimental model systems available to study these pathogenic CD8 T cells *in vivo*. However, the few studies that have been done characterizing the mechanisms used by CD8 T cells to induce CNS autoimmunity indicate that several of the paradigms of how CD4 T cells mediate CNS autoimmunity do not hold true for CD8 T cells or for patients with MS. Thus, myelin-specific CD4 T cells are likely to be one of several important mechanisms that drive CNS disease in MS patients. The focus of this review is to highlight the current models of pathogenic CNS-reactive CD8 T cells and the molecular mechanisms these lymphocytes use when causing CNS inflammation and damage. Understanding how CNS-reactive CD8 T cells escape tolerance induction and induce CNS autoimmunity is critical to our ability to propose and test new therapies for MS.

Keywords: multiple sclerosis, experimental autoimmune encephalomyelitis, T cells, tolerance, autoimmunity, central nervous system, MHC, TCR

MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is the most common neurological disease of young adults with over one million individuals worldwide afflicted by the disease (Noseworthy et al., 2000; Hafler et al., 2005; McFarland and Martin, 2007; Steinman, 2009). MS is an inflammatory disease of the central nervous system (CNS) that causes the demyelination of nerve cells and destroys oligodendrocytes, neurons, and axons (Lucchinetti et al., 2000; Frohman et al., 2006; Dutta and Trapp, 2007). Traditionally it has been thought that MS is an inflammatory disease primarily localized to the white matter of the brain and spinal cord. However, more recent studies have identified gray matter lesions in MS patients that appear at the earliest stages, accumulate over time, and exceed white matter lesions in progressive MS patients (Peterson et al., 2001; Bo et al., 2003; Calabrese et al., 2007; Lassmann et al., 2007; Fisher et al., 2008; Ontaneda et al., 2011). Within active MS lesions, the inflammatory immune cells are predominantly T cells and activated macrophages and microglia, and often form focal demyelinating plaques (Frohman et al., 2006; Lassmann et al., 2007). The targeting of the CNS by immune cells and the ensuing death of neuronal tissue causes a wide spectrum of disease pathologies in MS patients (Noseworthy et al., 2000; Keegan and Noseworthy, 2002; Hafler et al., 2005; Frohman et al., 2006). The disease course can be progressive with steadily increasing neurological deficits, or manifest as a relapsing–remitting disease, with discrete attacks of disease symptoms followed by periods of clinical stability (Steinman, 2009). The clinical signs of MS are highly variable. MS patients often have symptoms of upper motor neuron disease that include hyperreflexia, ataxia, spasticity, and visual defects. In some cases there is evidence of lower motor neuron disease such as sensory defects and partial or complete paralysis (Keegan and Noseworthy, 2002).

It is hypothesized that MS is a T cell mediated autoimmune disease. This hypothesis is primarily based on the genetic susceptibility of individuals for the disease, the presence of immune cells within active MS plaques and animal models of CNS autoimmunity mediated by T cells (McFarland and Martin, 2007; Steinman, 2009). The genetic susceptibility for MS is polygenic; however, the most prominent predisposing genetic element is genes within the major histocompatibility complex (MHC) locus (Fogdell-Hahn et al., 2000; Sospedra and Martin, 2005; Hafler et al., 2007; Olsson and Hillert, 2008; Sawcer et al., 2011). The MHC locus is a large gene cluster consisting of over 200 expressed genes. Due to extensive linkage disequilibrium it has been difficult to parse out the precise predisposing genes. In MS, it is now clear that the HLA-DR15 and HLA-DQ6 alleles of MHC class II have strong disease susceptibility association with MS (Hafler et al., 2007; Olsson and Hillert, 2008; Sawcer et al., 2011). These particular MHC alleles may be displaying particular sets of peptide antigens to autoreactive T cells (Wucherpfennig, 2001). In addition to MHC genes, polymorphisms in multiple immunologically relevant genes, including IL-7R and IL-2R, have been associated with MS susceptibility (Hafler et al., 2007; Sawcer et al., 2011). Therefore, one hypothesis has been that predisposing MHC class II alleles present particular disease relevant epitopes to dysregulated, pathogenic T cells. However, other MHC class II alleles can confer some protection from disease even when the susceptible MHC class II allele is expressed (Olsson and Hillert, 2008; Ramagopalan et al., 2009). This suggests that the role of MHC in autoimmunity is

likely to be multi-layered, involving functions from multiple gene products.

CD4 T cells specific for many CNS proteins, including myelin associated glycoprotein (MAG), myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP), can be isolated from MS patients and healthy individuals (Sospedra and Martin, 2005). These data, combined with evidence that MHC class II alleles confer the strongest genetic susceptibility, has suggested that in patients with MS, CNS protein-specific CD4 T cells become activated, cross the blood/brain barrier, and induce CNS autoimmunity (Sospedra and Martin, 2005; Steinman, 2009). Further support for this hypothesis comes from the many animal models of CD4 T cell mediated experimental autoimmune encephalomyelitis (EAE), that manifest clinical and pathological symptoms with many similarities to MS (Kuchroo et al., 2002; Ercolini and Miller, 2006; Goverman, 2009).

EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Animal models of CNS autoimmunity are primarily focused on the role of Th1 and Th17 phenotype CD4 T cell mediated pathology (Bettelli et al., 2007; Goverman, 2009). The majority of CNS proteins identified that can be targets of EAE-inducing CD4 T cells are components of compact myelin or proteins associated with myelin synthesis (Sospedra and Martin, 2005). The classical animal model of MS, CD4 T cell mediated EAE (CD4–EAE), is induced by immunizing animals with myelin proteins or peptides emulsified in complete Freund's adjuvant. This protocol predominantly induces Th1 and Th17 phenotype CD4 T cell responses, and is a poor elicitor of CD8 responses (Zamvil and Steinman, 1990; Cua et al., 2003). In addition, CD4–EAE is induced by transferring CNS-specific Th1 or Th17 CD4 T cells into naïve recipient animals (Zamvil and Steinman, 1990; Langrish et al., 2005; Lees et al., 2008; O'Connor et al., 2008; Jager et al., 2009). Similar to patients with MS, the disease course of CD4–EAE can be acute, relapsing/remitting, or chronic progressive. However, the disease pathologies associated with CD4–EAE are usually less diverse than the disease symptoms in MS patients (Steinman and Zamvil, 2005; Friese et al., 2006; Gold et al., 2006).

The major mouse models of EAE–MOG induced EAE in C57BL/6 mice, MBP induced EAE in B10.PL mice, and PLP mediated disease in SJL mice – present symptoms of ascending flaccid paralysis (Zamvil and Steinman, 1990; Vanderlugt and Miller, 2002). The disease begins with a loss of tail tone and can progress through an ascending paralysis affecting the rear legs, followed by the fore legs until the animals become morbid. These pathologies are reminiscent of MS patients with lower motor neuron disease (Keegan and Noseworthy, 2002). There are some models of CD4–EAE that present "atypical" axial-rotatory disease, characterized by mice that roll continuously or have severe deficiency in balance without paralysis (Greer et al., 1996; Sobel, 2000; Abromson-Leeman et al., 2007; Lees et al., 2008; Stromnes et al., 2008). Several studies have shown that the ratio of Th1 versus Th17, or the ability of CNS cells to respond to IFNγ signaling, contributes to the location of affected CNS area and/or the presentation of classical or atypical disease symptoms (Lees et al., 2008; Stromnes et al., 2008; Jager et al., 2009). The genetic background of mice that get CD4 T cell mediated atypical EAE include C57BL/6, Balb/c, and C3H

mice, genetic backgrounds that also develop classical ascending paralytic disease when immunized with different CNS antigens (Sobel, 2000). Thus, there are indications that the immunizing antigen, T cell trafficking and/or the phenotype of the CD4 T cells, and not necessarily the genetic background, may be determining factors in the induction of non-classical disease symptoms of EAE. Overall, although there are heterogenic disease pathologies induced by CD4 T cells, CD4–EAE still likely mimics some, but not all, aspects of CNS inflammation in MS.

Autoreactive CD4 T cells alone are unlikely to fully account for all of the effector mechanisms that drive the disease process in MS. Disease inducing, myelin-specific CD4 T cells within the CNS release the signature Th1 and Th17 cytokines. The release of cytokines and chemokines causes an increase in MHC expression within the CNS, leads to the activation of microglia, and induces the recruitment of monocytes and non-specific T cells to the area of inflammation (Prineas, 1979; Raine et al., 1984; Carson et al., 1999; Brabb et al., 2000; Krakowski and Owens, 2000; Juedes and Ruddle, 2001; Becher et al., 2006; Mildner et al., 2008). Several of these cytokines, including IFNγ, TNFα, IL-6, IL-12, and IL-23, have been directly implicated in disease pathogenesis (Krakowski and Owens, 1996; Okuda et al., 1998; Sean Riminton et al., 1998; Steinman, 2001; Cua et al., 2003; Deshpande et al., 2006). Using the CD4–EAE disease model as a screen, several therapies that ameliorate CD4–EAE have been developed for clinical trials to reduce symptoms in MS patients. While some of the treatments validated in the CD4–EAE model have reduced disease symptoms in MS patients, other therapies have not affected clinical symptoms, or have been detrimental to patients (Steinman and Zamvil, 2005; Friese et al., 2006; Gold et al., 2006). For example, the removal of CD4 T cells or TNFα from MS patients had little effect on disease symptoms, while both abolish disease in CD4–EAE models (Waldor et al., 1985; Llewellyn-Smith et al., 1997; van Oosten et al., 1997; Sean Riminton et al., 1998; Lenercept, 1999). Thus, CD4–EAE probably mimics only a subset of the events that occur during MS. The discovery of other key events in the pathogenesis of MS disease will be facilitated by animal models that mimic events not already evoked by CD4 T cells.

CD8 T CELLS IN MULTIPLE SCLEROSIS

The lymphocytes in MS plaques are biased toward the CD8 lineage. This bias occurs regardless of the stage of activity or disease, and can be as prominent as 10:1, CD8 versus CD4 T cells (Booss et al., 1983; Traugott et al., 1983b; Hauser et al., 1986; Babbe et al., 2000; Lassmann et al., 2007). At the margin of chronic and active lesions, CD8 T cells have been identified interacting with antigen presenting cells (APC; Serafini et al., 2006). Through normally poorly expressed, MHC class I proteins are highly expressed within the MS lesion on astrocytes, oligodendrocytes, and neurons, implying that CD8 T cells could be directly engaging these cell types as well (Traugott et al., 1983a; Wong et al., 1984a; Neumann et al., 1995, 1997). CD8 T cells with an activated/memory phenotype have been observed to be enriched within the CNS tissue and cerebrospinal fluid (CSF) of MS patients (Jacobsen et al., 2002; Junker et al., 2007), and histological analysis of some cases of acute MS have shown granzyme B-expressing CD8 T cells in close proximity or attached to oligodendrocytes or demyelinated axons

(Neumann et al., 2002; Serafini et al., 2006; Lassmann et al., 2007). Interestingly, Fugger and colleagues have found that a large percentage of CD8 T cells in acute and chronic MS lesions express IL-17 (Tzartos et al., 2008), a highly pro-inflammatory cytokine associated with pathogenic Th17 CD4 T cells and Tc17 CD8 T cells (Cua et al., 2003; Bettelli et al., 2007). This activated/memory phenotypic skewing of CD8 T cells within the CSF occurs even in early diagnosed MS patients. Furthermore, Brück and colleagues demonstrated that the number of CD8 T cells and macrophages within the lesions correlates with the extent of acute axonal damage, as defined by the accumulation of amyloid precursor protein (APP; Bitsch et al., 2000). In addition, genetic association studies have identified some MHC class I alleles, including HLA-A3, as a genetic susceptibility allele (Friese and Fugger, 2005; Sawcer et al., 2011). In contrast, the MHC class I allele, HLA-A*0201, has been shown to reduce the risk of MS in individuals that express the MHC class II proteins, HLA-DRB1*1501, and DQB1*06 (Fogdell-Hahn et al., 2000; Brynedal et al., 2007). These observations support the idea that CD8 T cells are involved in pathogenesis of MS, as active contributors to the development of MS lesions, and/or as protective regulatory T cell populations that limit disease.

Several groups have isolated T cells from the blood, CSF, and MS lesions (via microdissection), and analyzed these lymphocytes by complementarity determining region 3 (CDR3) spectratyping to determine the diversity of T cell clones. Initial studies by Babbe et al. (2000) demonstrated that the majority of CD8 T cells within an MS plaque were progeny of a few initial clones. One particular clone accounted for 35% of the CD8 T cells within an MS plaque. Later studies analyzing larger numbers of MS patients demonstrated that CD8 T cells isolated from CSF fluid also contained clonal expansions, some of which persisted for 5 years (Jacobsen et al., 2002; Skulina et al., 2004). To identify the antigen specificity of these CD8 T cells, paired TCRα, and TCRβ chains have been cloned and re-expressed in T cell lines (Seitz et al., 2006). However, the antigen specificity of these CD8 T cells has yet to be determined. Although the specificity of the CD8 T cells in MS plaques is unknown, their clonal nature suggests that they are responding against selected antigens.

Central nervous system-reactive CD8 T cells may be pathogenic in MS patients. Pioneering studies by Biddison and colleagues and Hafler and colleagues identified and analyzed CD8 T cells specific for several CNS proteins, including MAG, MBP, and PLP, from MS patients (Tsuchida et al., 1994; Dressel et al., 1997). These studies clearly demonstrated that MS patients carry a population of CD8 T cells specific for myelin proteins. These MAG-, MBP-, and PLP-specific CD8 T cells are capable of killing neuronal cells *in vitro* and release TNFα and IFNγ, suggesting that they may contribute to CNS disease, although the precise role of these cells in the pathogenesis of MS has not been fully elucidated (Tsuchida et al., 1994; Jurewicz et al., 1998; Medana et al., 2001). More recent analysis of peripheral blood suggests that MS patients carry CD8 T cells able to express IFNγ and TNFα in response to a multitude of CNS protein epitopes. Several studies have demonstrated that these CNS-reactive CD8 T cells were found in greater frequency and bore an activated/memory phenotype in MS patients, suggesting they had been activated within the patient by CNS antigens (Crawford et al., 2004; Zang et al., 2004; Niland et al., 2005).

In contrast, these same T cells isolated from control subjects displayed a naïve T cell phenotype. Differences in the frequency of CNS antigen-specific CD8 T cells between MS patients and control subjects have not always been observed, as myelin-reactive CD8 T cells have been identified in healthy subjects as well (Berthelot et al., 2008). This group of studies clearly indicates that individuals carry a population of CD8 T cells specific for a variety of CNS antigens. The presence of CNS-reactive T cells exhibiting an activated/memory phenotype in MS patients and the oligoclonal nature of CD8 T cells within MS plaques suggest that CD8 T cells are active participants within the destructive CNS immune response.

In addition to a pathogenic role for CD8 T cells in MS, regulatory CD8 T cells may contribute to limiting disease severity or occurrence in MS patients. Early experiments demonstrated that the suppressor function of CD8 T regulatory cells in MS patients may be defective as compared to healthy individuals (Antel et al., 1986). Consistent with these findings, a specific CD8 T cell clone has been shown to regulate MBP-specific CD4 T cells (Chou et al., 1992). CD8 T cells that can lyse myelin-specific CD4 T cells have been detected in MS patients, and vaccination of MS patients with irradiated myelin-specific CD4 T cells elicited CD8 T cells that could specifically kill these CD4 T cells (Zhang et al., 1993; Correale et al., 2000). More recently, longitudinal magnetic resonance imaging (MRI) analysis has shown a negative correlation between the percentage of Tc2 cytokine-producing CD8 T cells in the periphery of MS patients and the development of lesions (Killestein et al., 2003). Furthermore, the beneficial effects of treating MS patients with glatiramer acetate may in part be due to activating regulatory CD8 T cells (Tennakoon et al., 2006). In addition to these, and many other studies of human CD8 T regulatory cells, mouse models have also shown potent disease modifying effects of CD8 T regulatory cells through the secretion of IL-10 and other soluble mediators, the regulation of APC function, as well as by eliminating activated CD4 T cells by CD8 T cells via recognition of the non-classical Qa-1 MHC molecule (Jiang and Chess, 2006; Goverman, 2009).

ANIMAL MODELS OF CD8 T CELL MEDIATED CNS AUTOIMMUNITY

Several years ago we and others started developing CD8 T cell models of CNS autoimmunity. At the time, MS was generally believed to be primarily a CD4 T cell mediated autoimmune disease. However, because MS plaques contain a large excess of CD8 T cells relative to CD4 T cells, we were concerned that a major lymphocyte population within the inflamed site was being under studied. Because of the cytotoxic and pro-inflammatory nature of CD8 T cells, we hypothesized that myelin-specific CD8 T cells contribute to CNS autoimmunity. Our studies focused on whether MBP-specific CD8 T cells could induce CNS autoimmunity. Because MBP is a self protein, we were concerned that CD8 T cells specific for MBP might be subject to immune tolerance mechanisms. To avoid tolerance issues, we initially isolated MBP-reactive CD8 T cells from C3H MBP-deficient *shiverer* (C3H MBP$^{-/-}$) mice (Huseby et al., 1999). These studies demonstrated that MBP-specific CD8 T cells, similar to several CD4 T cell epitopes of MBP and PLP, are subject to immune tolerance (Goverman, 2011). However, some

MBP-specific CD8 T cells are present in the peripheral repertoire of wild-type C3H mice. Furthermore, the activation of MBP-specific CD8 T cells in animals induces severe, demyelinating CNS autoimmunity (Huseby et al., 2001a).

The pathology of MBP-specific CD8 T cell mediated disease had many similarities to some human MS patients (Lucchinetti et al., 2000), including the presence of lesions throughout the brain (Huseby et al., 2001a). Histological analysis of diseased brains indicated the lesions were vascular in nature, involving capillaries and venules, and consisted of perivascular cuffing. Vacuolation of the surrounding nervous tissue was common and consisted of demyelination and cytoplasmic swelling. In contrast to many typical CD4–EAE models, this MBP-specific CD8 T cell mediated disease model had a predominance of lesions in the brain instead of the spinal cord, displayed a general lack of inflammation (except that which was directly associated with vascular walls), and showed severe demyelination and perivascular cell death, suggesting a cytotoxic or ischemic injury. Antibody blocking experiments demonstrated a role for IFNγ in contributing to disease severity (Huseby et al., 2001a). Using TCR Tg mice that generate MBP-specific CD8 T cells, Goverman and colleagues have gone on to show how endogenous MBP influences this T cell repertoire (Perchellet et al., 2004, 2008), and have demonstrated that viruses can induce CD8 T cell-mediated autoimmunity by breaking peripheral tolerance mechanisms (Ji et al., 2010).

CD8 T cell lines reactive to the MOG derived peptide, MOG_{35-55}, can induce chronic CNS autoimmunity in C57BL/6 mice (Sun et al., 2001; Ford and Evavold, 2005). Unlike the disease symptoms induced by MBP-specific CD8 T cells, these MOG-reactive CD8 T cell lines induced ascending flaccid paralysis, and caused lesions in both the spinal cord and brain. The reason for the differences in disease symptoms between the MBP and MOG models of CD8–EAE is unknown. The differences could be due to the CD8 T cells targeting different proteins or due to differences between the C3H and C57BL/6 genetic backgrounds.

Humanized mouse models have been used to study CD8 T cell responses targeting myelin epitopes presented by human HLA-A molecules. CD8 T cells targeting $MOG_{181-189}$, presented by the MHC class I allele, HLA-A*0201, were observed to potentiate an autoreactive CD4 T cell response by accelerating and worsening the encephalitogenic process (Mars et al., 2007). Friese et al. (2008) have more recently generated a humanized mouse model of MS in which an MHC class I-restricted TCR specific for PLP_{45-53} bound to HLA-A*0301, isolated from an MS patient, is expressed in mice which also express the MHC Class I molecule HLA-A*0301. These mice, constructed on a C57BL/6 genetic background, develop a low grade spontaneous MS-like disease which becomes more severe upon immunization with PLP peptides. Following immunization with PLP peptides, there are two phases of disease, an early infiltration that is dominated by CD8 T cells and late disease that requires MHC class II expression. Coincident with the late disease is the expansion within the CNS of CD4 T cells reactive to MOG_{35-55}. These experiments nicely demonstrate that T cells expressing a human MHC class I-restricted TCR specific for a CNS protein can be pathogenic and can induce epitope spreading to the CD4 T cell compartment. When the human TCR Tg mice were crossed onto mice expressing HLA-A 0301 and the protective MHC class I

allele, HLA-A*0201, thymocytes expressing this PLP-specific TCR were subject to elimination by negative selection. T cells expressing the PLP_{45-53}-specific TCR did not recognize the PLP_{45-53} epitope bound to HLA-A*0201, suggesting the elimination of these pathogenic T cells was not induced by recognizing endogenous PLP (Friese et al., 2008). The induction of negative selection in mice expressing HLA-A*0201 support the hypothesis that one of the protective effects of specific MHC class I alleles is the elimination of pathogenic T cells during development.

Complementing the active induction models are a set of mice which spontaneously succumb to CD8 T cell-dependent CNS autoimmunity. Fournier and colleagues observed that transgenic C57BL/6 mice, in which the co-stimulatory molecule CD86 is constitutively expressed on peripheral T cells and resident CNS microglia, succumb to a CD8 T cell-dependent CNS demyelinating disease (Brisebois et al., 2006). CNS infiltration by CD8 T cells results in mice which display a deterioration of hind-limb control and coordination, weak tail movement, and weight loss. The age at which these mice succumb to spontaneous disease is younger in both MHC class II-deficient mice as well as CD4-deficient mice, suggesting the pathogenic CD8 T cells are being regulated by a CD4 T cell populations. The specificity of these pathogenic CD8 T cells has yet to be determined. The non-paralytic disease symptoms and pathologies in CD86 transgenic mice are similar to the MBP-specific CD8 T disease model in C3H mice, while differing from the paralytic disease mediated by MOG_{35-55} specific CD8 T cells. Two additional models have recently been observed in which pathogenic CD8 T cells induce both clinical signs of CNS disease as well as demyelination. Mice over-expressing PLP within oligodendrocytes of the CNS developed progressive clinical signs of neurological damage including ataxia, tremors, and seizures after 12–18 months of age that was dependent up CD8 T cells (Ip et al., 2006). CD8 T cells and macrophages accumulated within the brains of these mice, with a limited B cell and CD4 T cell component. Affected areas of the CNS included the optic nerve, cerebral white matter, and spinal cord. Mice in which the oligodendrocytes are deficient in peroxisome show a similar axonal loss and neuroinflammation as the PLP over-expressing mice (Kassmann et al., 2007). The antigen specificity of the CNS infiltrating CD8 T cells remains unknown in both models. These spontaneous models, along with the active induction models, further suggest that CD8 T cells can induce a range of different disease manifestations, and clearly demonstrate that CNS-specific CD8 T cells are present in the peripheral T cell repertoire and when activated, can induce CNS autoimmunity.

CELLULAR TARGETS OF PATHOGENIC CD8 T CELLS

The cellular target of pathogenic myelin-specific CD8 T cells *in vivo* is unknown. Normally, there is very little MHC class I expression in the CNS. Neurons and oligodendrocytes express only low levels of MHC class I, constitutively. Astrocytes, microglia, blood vessel endothelial cells, and bone marrow derived-APC (BM-APC) resident within the CNS do express MHC class I constitutively, though none of these cells synthesize myelin antigens (Traugott et al., 1983a; Wong et al., 1984a; Massa et al., 1993; Neumann et al., 1995, 1997; Hoftberger et al., 2004; Frohman et al., 2006). However, BM-APC, and to a lesser extent, blood vessel

endothelial cells, can cross-present exogenously synthesized proteins on MHC class I proteins (Limmer et al., 2000; Rock and Shen, 2005; Galea et al., 2007). Thus, the initial recognition of myelin peptides presented on MHC class I proteins is likely by BM-APC or blood vessel endothelial cells. Whether CD8 T cells can directly lyse oligodendrocytes *in vivo* is unknown. MHC expression by oligodendrocytes does increase after inflammation or in response to IFNγ, therefore it may be possible for CD8 T cells to directly target oligodendrocytes once inflammation begins (Wong et al., 1984a,b; Tsuchida et al., 1994; Neumann et al., 1997; Hoftberger et al., 2004). Thus, it is not surprising that when we and others neutralized IFNγ, the severity of CD8–EAE disease is drastically reduced (Huseby et al., 2001a; Brisebois et al., 2006).

To begin to identify which CNS cell types could be targets of CD8 T cells, several groups have created transgenic mice which express neo-self antigens in specific cell populations. The first of these models, created by the Oldstone group, expressed the lymphocytic choriomeningitis virus (LCMV) glycoprotein under the MBP promoter (Evans et al., 1996). These mice expressed very low levels of the LCMV protein exclusively in the brain, likely in oligodendrocytes. Following two subsequent viral infections, focal areas of myelin degradation were observed, and expression of both MHC class I and class II molecules was found on oligodendrocytes. These data suggested that CD8 and/or CD4 T cells may interact directly with oligodendrocytes and play a role in oligodendrocyte injury. More recently, two sets of mice expressing either ovalbumin (OVA) or the influenza virus hemagglutinin (HA) proteins selectively within oligodendrocytes, have been constructed and analyzed.

When mice expressing OVA selectively in oligodendrocytes (ODC–OVA) were genetically crossed with OT-1 mice, transgenic mice expressing a TCR specific for OVA$_{257-264}$ presented by H2-K^b, the double transgenic mice succumbed to an extremely aggressive, lethal fulminant demyelinating CNS autoimmunity (Na et al., 2008). Lesions within the cerebellum, brainstem, optic nerve, and spinal cord were observed. *In vitro*, OVA-transgenic oligodendrocytes were capable of activating naïve OT-I cells. The potency of OVA-expressing oligodendrocytes to activate OT-I CD8 T cells *in vitro* was further amplified by the addition of exogenous IFNγ to the cultures. The secretion of IFNγ from OT-1 T cells was also found to be required for the loss of oligodendrocytes, as well as axon damage within the CNS. In contrast to this model, Balb/c mice expressing the HA protein in oligodendrocytes (MOG–HA) did not develop spontaneous autoimmunity when crossed with transgenic mice expressing an HA-specific TCR, Cl4, that recognizes HA$_{512-520}$ presented by H2-K^d (Saxena et al., 2008). In these mice, the HA-specific CD8 T cells maintained ignorance to the neo-self protein. However, transfer of *in vitro* activated HA-specific CD8 T cells induced weight loss, demyelination, and reduced mobility, but not paralysis. The inflammatory lesions were primarily within the spinal cord and optic nerve. HA-specific CD8 T cells within the CNS were found in close apposition with oligodendrocytes and some had polarized Granzyme B containing granules toward the oligodendrocytes (Saxena et al., 2008). The loss of oligodendrocytes prior to demyelination suggests these CD8 T cells are directly killing these cells. Interestingly, when these same HA-specific CD8 T cells are transferred into mice expressing

the HA protein selectively in astrocytes, the mice developed a monophasic brain inflammation with an absence of any clinical signs of disease (Cabarrocas et al., 2003). Combined, these data suggest that CD8 T cells, which have a strong reactivity for an epitope that is processed and presented well on MHC class I molecules, can directly target oligodendrocytes within the CNS. The differences in disease course and severity among the transgenic neo-self models of CD8 T cell mediated CNS autoimmunity further suggests that a multitude of factors, including target antigen expression level, CNS cell type expression, TCR–pMHC affinity, and genetics, likely contribute to whether a CD8 T cell response is pathogenic.

WHY ARE PATHOGENIC CNS-REACTIVE CD8 T CELLS PRESENT WITHIN THE MATURE T CELL REPERTOIRE?

The T cell mediated autoimmune nature of MS and EAE indicates that T cell tolerance of CNS proteins is incomplete. During T cell development, TCR undergo somatic recombination of their peptide and MHC binding CDR3 loops, as well as TCRα/β chain pairing (Davis and Bjorkman, 1988). The immense TCR diversity generated during T cell development allows the adaptive immune response to be able to generate specific T cell responses to unknown pathogens. This process, however, also creates self-reactive T cells. Many of these self-reactive T cells are purged during negative selection, due to the expression of the self protein within the thymus by dendritic cells or medullary thymic epithelial cells (Kappler et al., 1987; Kisielow et al., 1988; Mathis and Benoist, 2004; Kyewski and Klein, 2006). However, some self-reactive T cells that escape negative selection are then subject to peripheral tolerance mechanisms (Zheng and Rudensky, 2007). Autoimmune diseases arise when failures of thymic and peripheral tolerance mechanisms allow self-reactive T cells to be activated and attack healthy tissues. It has been clearly established that endogenous MBP induces some tolerance to the CD4 and CD8 T cell repertoire (Goverman, 2011). PLP also appears to induce some tolerance to the CD4 T cell repertoire specific for it (Kuchroo et al., 2002). Because MBP and PLP are expressed at extremely high levels within the CNS and some isoforms of these proteins are expressed within the thymus (albeit at extremely low levels by medullary thymic epithelial cells), it is not surprising some T cell tolerance occurs (Kyewski and Klein, 2006). Negative selection can also occur to T cells specific for CNS proteins not expressed in the thymus. Our studies of CD4 T cells specific for MBP$_{121-140}$, an epitope that is not expressed in the thymus, indicate that BM-APC can acquire MBP from exogenous sources and cause the elimination of developing T cells (Huseby et al., 2001b). The extremely low level of MOG (around 1,000–5,000 times less abundant than MBP and PLP within the CNS) does not appear to induce significant tolerance to the CD4 T cell repertoire (Delarasse et al., 2003). It has not been determined whether other myelin proteins induce CD4 T cell tolerance, nor is it known whether CNS proteins other than MBP induce tolerance to the CD8 T cell repertoire.

There are three main hypotheses for how pathogenic myelin-reactive T cells escape tolerance induction in the thymus, primarily derived from studies of CD4 T cell responses: (1) T cells specific for exons of myelin proteins neither expressed nor presented within the thymus fail to be deleted because the antigen is not presented to

developing thymocytes (Kuchroo et al., 2002; Kyewski and Klein, 2006). (2) For myelin proteins that are presented well in the thymus, central tolerance eliminates thymocytes expressing TCR that have a strong reactivity for these myelin antigens while allowing thymocytes expressing TCR which have a weak reactivity to develop (Goverman, 2011). Thymocytes that escape this form of central tolerance may express TCR with a low affinity for myelin epitopes bound to self-MHC proteins, or express TCR that bind myelin epitopes bound to self-MHC with unconventional binding modes (Wucherpfennig et al., 2009). (3) Autoreactive T cells specific for myelin proteins that are expressed in the thymus but are poorly presented (e.g., peptide sequences that bind MHC proteins poorly) may not be deleted because the amount of MHC + peptide complex in the thymus is very low (Goverman et al., 1993; Liu et al., 1995). Myelin-specific T cells expressing low affinity TCR, TCR that bind pMHC with unconventional bind modes, or TCR that target epitopes that are poorly presented may be pathogenic because the expression level of the myelin protein is much higher in the CNS than thymus, and thus APC within the CNS can present enough MHC + peptide complexes to activate these lymphocytes.

Do these models of T cell tolerance to myelin antigens hold true for CD8 T cells as well? Though little is currently known about how CNS-reactive CD8 T cells escape tolerance induction, it was very surprising that some CD8 T cells with a strong reactivity for MBP$_{79-87}$ were allowed to develop and seed the mature T cell repertoire (Huseby et al., 2001a). This myelin epitope is expressed within the thymus and most T cells with a strong reactivity for this epitope are eliminated. Using a completely novel mechanism to escape tolerance induction, Perchellet et al. (2004) found that MBP$_{79-87}$-specific CD8 T cells could avoid both central and peripheral deletion by removing H2K^k–MBP$_{79-87}$ complexes from APC without proliferating. Thus, the major form of immune regulation that governs myelin-specific CD4 T cells may not fully explain the development and control of CNS-reactive CD8 T cells. To understand why CNS autoimmunity arises, it will be important to identify the pathways in which CNS-reactive CD4 and CD8 T cells are normally eliminated or regulated, and why these processes fail in patients with MS. Overall, how similar or different the contribution of CNS-reactive CD4 versus CD8 T cells to the disease pathologies of MS is just beginning to be understood.

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