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Control of Experimental Autoimmune Encephalomyelitis by CD4+ Suppressor T Cells: Peripheral versus in situ Immunoregulation

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Abstract

The pathogenesis of experimental autoimmune encephalomyelitis (EAE) can be efficiently kept under control by specialized subsets of CD4+ T lymphocytes able to negatively regulate the function of T cells with encephalitogenic potential. A number of observations support a role for such suppressor T cells in controlling early phases of disease development at the level of peripheral lymphoid organs but there is also evidence suggesting immunoregulation within the central nervous system (CNS) microenvironment itself. This review evaluates the sites of regulation based on available data from distinct experimental models. We then discuss these aspects with reference to suppressor CD4+ T cells induced through the epicutaneous application of pure CNS antigens that confer long term protection against EAE. Finally, we give an overview of genes recently discovered to be important in regulation of the immune system that may also prove to be key players in the modulation of EAE and MS.

Keywords

EAE; Tolerance; Suppressor T cells; Autoimmunity; Epicutaneous Immunization

1. Introduction

Experimental autoimmune encephalomyelitis (EAE) is a pro-inflammatory autoimmune disorder targeting the central nervous system (CNS) that serves as an animal model for the human disease multiple sclerosis (MS). MS is characterized by an attack on the white matter of the CNS by components of the immune system (Goverman and Brabb, 1996; Goverman et al., 1997; Steinman, 1992). During EAE, myelin reactive CD4+ T lymphocytes that are predominantly of the T helper (Th) 1 subtype infiltrate the spinal cord and brain and, in concert with other infiltrating mononuclear cells such as macrophages, cause inflammation, oligodendrocyte cell death, demyelination, axonal degeneration and progressive ascending paralysis.

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It was shown long ago that the pathogenesis of EAE can be controlled by T cells with suppressor function. Pioneering experiments performed with guinea pigs, rats and mice have demonstrated that the subcutaneous injection of purified myelin antigens (or even brain extracts) emulsified in incomplete adjuvant (IFA) can prevent EAE induction (reviewed in (Lenz and Swanborg, 1999). A central role for mature T cells in such protection was indicated by the observation that lymph node, but not thymic, T cells isolated from disease resistant animals could confer protection to syngeneic recipients upon adoptive transfer (Swanborg, 1972; Swierkosz and Swanborg, 1975). Thus, immunization with autoantigen under non-encephalitogenic conditions can induce T cells with the capacity to suppress the development of the pro-inflammatory autoimmune disorder EAE, and the suppressive character of such T cells is dominant.

The demonstration that the pathogenic potential of CNS antigen specific CD4+ T cells could be controlled by naturally occurring suppressor T cells is more recent. The first solid evidence for suppression of T cells with encephalogenic potential by naturally occurring suppressor lymphocytes came from a study by Lafaille and colleagues (Lafaille et al., 1994). In this study, transgenic (Tg) mice expressing a MHC class II restricted αβ T cell receptor (TCR) specific for the myelin basic protein (MBP)1-9 epitope did not show a substantial incidence of spontaneous disease unless they were crossed to mutant mice unable to produce endogenously rearranged antigen receptors (RAG-1 deficient mice) to derive mice with all T cells expressing only the transgenic αβ TCR. Under such conditions, 100% of MBP TCR Tg RAG deficient mice rapidly developed spontaneous EAE, indicating that MBP1-9 specific T cells are efficiently controlled by other lymphocytes in mice that are not deficient in RAG-mediated functions. Subsequent studies taking advantage of mice with various engineered mutations led to the conclusion that the protection against spontaneous EAE was due to immunoregulation mediated by suppressor CD4+ αβ T cells, because TCR Tg mice lacking only B cells, γδ T cells, CD8+ T or NK T cells remained free of disease. Moreover, the transfer of purified mature CD4+ T cells from unmanipulated syngeneic donors was sufficient to protect TCR Tg RAG deficient mice from developing spontaneous EAE (Olivares-Villagomez et al., 1998; Van de Keere and Tonegawa, 1998). This effect was not associated with conversion of endogenous T cells to a suppressive status by the transferred T cells (Hori et al., 2002). The antigen receptor repertoire of these αβ TCR $CD4^+$ suppressor T cells could be diverse or relatively restricted yet the specificity of their TCR directed their suppressive function (Olivares-Villagomez et al., 2000). Finally, while interleukin (IL)-4 appeared not to play a role, IL-10 partially contributed to the protection against spontaneous EAE conferred by naturally occurring $CD4^+$ suppressor T cells in this model.

The same year, Lafaille and colleagues published their seminal article, it was reported that the oral administration of MBP could protect SJL mice from EAE by inducing peripheral tolerance (Chen et al., 1994). CD4+ T cells isolated from the mesenteric lymph nodes of protected mice produced predominantly transforming growth factor (TGF)-β and suppressed EAE induced with MBP or proteolipid protein. These findings indicated that mucosally-induced CD4+ suppressor T cells can control EAE pathogenesis (Weiner, 2001a; Weiner, 2001b; Wu and Weiner, 2003).

2. Suppression of EAE by $CD25^+CD4^+$ T cells

$CD25^+CD4^+$ T cells, commonly referred to as regulatory T cells (Treg cells) represent a now-well-characterized, naturally-arising subset of $CD4^+$ T cells with efficient suppressive function. Most CD25+CD4+ T cells are of thymic origin and suppress immune responses to self-antigens. They are found both in humans and rodents and constitute between 5–10% of the circulating CD4+ T cell pool under physiological conditions. Treg cells were first described by Sakaguchi et al. who showed that the removal of the thymus in neonates resulted in systemic

autoimmunity due to deficiency in this T cell subset (Sakaguchi et al., 1995). Transfer of $CD25^+CD4^+$ T cells from syngeneic animals protected these same thymectomized neonates from autoimmune diseases. Since these initial findings, a plethora of studies have demonstrated that $CD25^+CD4^+$ T cells suppress immune responses directed to self-tissues during/following microbial infections and can mediate transplantation tolerance. They display features of functionally inactivated T cells and are capable of transferring this status of anergy to other T cells both in vivo and in vitro. $CD25^+CD4^+$ T cell-mediated suppression requires contact with effector T cells at least in vitro. $CD25^+CD4^+$ T cells are able to express elevated levels of cytotoxic T-lymphocyte antigen-4 (CTLA-4) and glucocorticoid-induced tumor necrosis factor receptor (GITR) relative to their naive counterparts. Under certain experimental situations, TGF-β was found to be involved in $CD25^+CD4^+$ T cell dependent suppression. Most remarkably, $CD25^+CD4^+$ T cells consistently express the fork-head transcription factor FoxP3, which is central to their natural emergence and function in vivo. Their peripheral maintenance is highly dependent on IL-2. There exist recent and comprehensive reviews on Treg cells (Kim and Rudensky, 2006; Li et al., 2006; Picca et al., 2006; Sakaguchi, 2004; Sakaguchi, 2005; Sakaguchi et al., 2006); therefore their characteristics will not be further described here.

Upon adoptive transfer to syngeneic recipients, $CD25^+CD4^+$ T cells purified from C57BL/6 mice confer protection against active EAE induced 3 days later by immunization with the immunodominant epitope of myelin oligodendrocyte glycoprotein (MOG35–55) in the presence of complete Freund's adjuvant (CFA) (Kohm et al., 2002). In vitro, $CD25^+CD4^+$ T cells inhibit the proliferation of cytokine production by MOG35–55 specific Th1 cells. Upon cotransfer, $CD25^+CD4^+$ T cells can also attenuate the severity of EAE induced by previously activated, blasting, $MOG_{35–55}$ specific T cells. In this case, interferon gamma (IFN-γ) and tumor necrosis alpha (TNF-α) producing cells were still activated and found at similar numbers in the recipients at the peak of disease, but there was an increased frequency of $MOG_{35–55}$ specific T cells harboring a Th2 profile (secretion of IL-4/IL-5). CNS infiltration by $CD4^+$ T cells also decreased, suggesting that $CD25^+CD4^+$ T cells protect against EAE progression through enhanced anti-inflammatory cytokine secretion and inhibition of CNS inflammation. Of interest was the fact that $CD25^+CD4^+$ T cells showed an enhanced capacity to migrate to lymph nodes perhaps due to increased expression level of intercellular adhesion molecule-1 (ICAM-1) and P-Selectin. Upon histopathological analyses, the brain and spinal cord of recipients did not contain detectable levels of adoptively transferred $CD25^+CD4^+$ T cells.

In a genetically distinct model of EAE, disease severity can be enhanced when the pool of $CD25^+CD4^+$ T cells is reduced. Thus, immunization of female SJL mice with the 139-151 fragment of myelin proteolipid protein (PLP) after in vivo depletion of $CD25^+CD4^+$ T cells leads to exacerbation of clinical and histologic features and an altered cytokine profile by LN cells upon specific challenge in vitro (increased IFN-γ secretion and decreased IL-10 secretion) (Zhang et al., 2004). In line with this, $CD25^+CD4^+$ T cells purified from normal SJL mice secreted high levels of IL-10 upon stimulation in vitro, and their transfer to unmanipulated SJL recipients suppressed EAE. In this study, IL-10 appeared clearly involved in the suppressive activity of $CD25^+CD4^+$ T cells in vitro and in vivo. For instance, unlike $CD25^+CD4^+$ T cells from wild type C57BL/6 mice, purified $CD25^+CD4^+$ T cells isolated from IL-10 deficient C57BL/6 mice could not interfere with the development of $MOG_{35–55}$-induced EAE. Depletion of $CD25^+$ cells prior to EAE induction also caused a more severe disease in the C57BL/6-$MOG_{35–55}$ model, and $MOG_{35–55}$ responsive T cells displayed enhanced IFN-γ production (Montero et al., 2004).

The long term control of MBP specific naive T cells by $CD25^+CD4^+$ T cells can involve the induction of a state of tolerance, characterized by a repression of IFN-γ production, which does not prevent cells from undergoing activation or trafficking to the CNS (Cabbage et al., 2007). Presumably, both IL-10 and TGF-β are involved in the protection mediated by such tolerized

T cells. In agreement with this, the confrontation with their cognate ligand presented on activated antigen presenting cells (APCs) caused MBP specific tolerized T cells to limit their production of Th1 cytokines and to increase the transcription level of anti-inflammatory cytokine IL-10 and TGF-β. The maintenance of this anti-inflammatory profile in response to self antigen on activated APCs depended on the continuous presence of $CD25^+CD4^+$ suppressor T cells (Cabbage et al., 2007).

As for induced $CD25^+CD4^+$ T cells, an aggregated form of $PLP_{139–151}$ can convert specific naïve T cells to T cells producing IL-10 and expressing CD25, CTLA-4 and FoxP3 (Yu et al., 2005). These induced Treg cells could substantially ameliorate $PLP_{139–151}$-induced active EAE in SJL or (SJL x C57BL/6)F1 mice. Altogether, these observations indicate that $CD25^+CD4^+$ suppressor T cells can effectively control CNS antigen specific $CD4^+$ T cells with pathogenic potential.

3. Suppression of EAE by $CD25^-CD4^+$ T cells

It was soon realized that $CD25^+CD4^+$ T cells were not the only suppressor T cell subset able to control EAE development. CD25 negative $CD4^+$ T cells can also efficiently suppress $CD4^+$ T cells with encephalitogenic potential in vivo. This is the case for naturally-occurring suppressor $CD4^+$ T cells that prevent disease in the MBP TCR transgenic system (Lafaille et al., 1994). As mentioned above, the transfer of CD4+ T cells from syngeneic donors protects TCR Tg RAG-/- recipients from spontaneous EAE (Olivares-Villagomez et al., 1998; Van de Keere and Tonegawa, 1998). Purified $CD4^+$ T cells able to confer life-long protection from EAE upon transfer did not need to include T cells with surface expression of CD25 to exert such a suppressive effect. This clearly indicates that endogenous suppressor T cells other than $CD25^+CD4^+$ T cells could control MBP specific $CD4^+$ T cells.

Additional evidence for efficient EAE regulation by $CD25^-CD4^+$ T cells comes from a model of tolerance induction that relies on the repeated intranasal administration of CNS antigen peptides. Inhalation of Ac1-11 peptide, or its variants, prior to disease induction can lead to protection against EAE in H-2u mice (Anderton and Wraith, 1998; Metzler and Wraith, 1993). Such an approach has been successful in attenuating EAE even in a mouse model where virtually all T cells were specific (due to transgene driven TCR expression) for the relevant auto-antigen (Burkhart et al., 1999). In this case, the induced suppressor T cells appeared anergic, expressed CTLA-4 but remained CD25 negative. Moreover, their suppressive activity in vivo was critically dependent on IL-10 (Sundstedt et al., 2003). Further analysis demonstrated that $CD4^+$ suppressor T cells induced upon intranasal immunization did not express Foxp3, and their generation was independent of endogenous $CD25^+CD4^+$ T cells (Nicolson et al., 2006).

4. Control of encephalitogenic T cells by suppressor T cells within the CNS

The corpus of data mentioned above is, for the most part, compatible with a model where suppressor T cells can act in lymph nodes to interfere with the priming of CNS antigen reactive T cells, and therefore, the activation phase of the disease. In particular, the presence of infused $CD25^+CD4^+$ T cells that interfere with EAE pathogenesis in recipients, was overt in lymph nodes but not detected within the CNS (Kohm et al., 2002). In addition, the transfer of purified $CD4^+$ T cells that can protect young MBP TCR Tg RAG deficient mice from developing spontaneous disease had only a marginal effect in already diseased recipients, suggesting they may act primarily by interfering with the priming of MBP specific T cells.

There is evidence, however, suggesting that regulation of encephalitogenic T cells by suppressor T cells may also occur within the microenvironment of the CNS. For instance, $CD25^+CD4^+$ T cells with expression of CTLA-4, GITR and FoxP3 (i.e., canonical Treg cells)

can be found within the spinal cord of C57BL/6 mice during the recovery phase that follows $MOG_{35–55}$-induced EAE (McGeachy et al., 2005). The percentage and number of CNS $CD25^+CD4^+$ T cells increased as EAE progressed from the peak of disease through recovery. These T cells expressed CD103/αEβ7, an integrin hypothesized to facilitate the efficient migration of $CD25^+CD4^+$ T cells to sites of inflammation (Huehn et al., 2004). CNS $CD25^+CD4^+$ T cells produced IL-10 and functioned as potent suppressors in standard in vitro assays as well as (upon adoptive transfer) in vivo. During recovery, suppression did not appear to cause the physical elimination of CNS residing, IFN-γ producing CD25-$CD4^+$ T cells. Finally, the antibody-mediated depletion of CD25+ cells prior to EAE induction markedly delays recovery, while post-recovery depletion of $CD25^+$ cells abolishes the resistance to disease reinduction. These findings all support an important role for $CD25^+$ T cells in disease suppression. Thus, suppressor T cells appear able to operate within the CNS during the resolution of induced EAE in C57BL/6 mice, that is, during the control of ongoing inflammatory lesions. This control is likely to involve IL-10, because CNS resident $CD25^+CD4^+$ T cells produced IL-10. IL-10 production is a major factor for full recovery, because IL-10-/- mice develop severe EAE with no recovery from disease and IL-10 transgenic mice are resistant to EAE induction (Bettelli et al., 1998). IL-10 might indeed be central to suppression of EAE pathogenesis by $CD25^+CD4^+$ T cells since it is also involved in protection from EAE upon adoptive transfer of $CD25^+$CD4+ T cells (Zhang X et al., 2004). Furthermore, IL-10 transcript expression is upregulated in the CNS during the recovery phase (Samoilova et al., 1998) and IL-10 produced by PLP specific T cells can suppress EAE upon adoptive transfer to PLP peptide immunized mice (Mathisen et al., 1997).

The demonstration that suppressor T cells can control pathogenic T cells within the CNS might in fact help to explain previous observation of central tolerization, namely the in situ control of MBP reactive naïve T cells that can traffic to the CNS. For instance, naïve T cells can be found within the CNS regardless of whether they react to CNS antigens such as myelin epitopes (Brabb et al., 2000). CNS antigen specific and non-specific T cells found in the CNS are both phenotypically naïve when examined ex vivo. However, while T cells specific for CNS unrelated antigen were fully responsive to in vitro stimulation, CNS antigen-specific T cells did not respond to cognate stimulation. Such in situ control could involve various mechanisms and multiple cell types. It is striking to note that mononuclear cells isolated from the CNS of MBP TCR Tg mice can suppress the response of functional MBP specific T cells in vitro, whereas mononuclear cells isolated from the CNS of non-transgenic syngeneic mice cannot. The latter finding is compatible with (but does not prove) a role for tolerized MBP specific T cells in in situ immunoregulation. Finally, in very early experiments, the protection induced in rats by the subcutaneous delivery of MBP emulsified in incomplete adjuvant was characterized by a perivascular lymphocytic infiltration in the CNS, indicating that there was suppression of clinical, but not histologic, EAE (Swierkosz and Swanborg, 1975). This observation is also suggestive of immunosuppression occurring in situ, at least partially.

5. Suppressor T cells induced via epicutaneous immunization with pure CNS antigens

Because the epicutaneous application of protein antigens can induce marked Th2 immune responses in mice (Herrick et al., 2000; Wang et al., 1996; Wang et al., 1999), we have tested the ability of this route of autoantigen delivery to impact on the development of EAE. We initially used transgenic mice with most T cells expressing a TCR specific for the N-terminal peptide (Ac1-11) of MBP (MBP TCR Tg mice) (Hardardottir et al., 1995; Lafaille, 2004). We performed epicutaneous immunization (ECi) with pure Ac1-11 only. In this approach, Ac1-11 loaded on an adhesive patch is applied to the shaved skin of mice for two weeks prior to disease induction (Ac1-11 peptide emulsified in CFA). We observed significant protection from both a spontaneous form of EAE (i.e. MBP TCR Tg mice when devoid of any T cells with

endogenously produced TCR)(Lafaille et al., 1994) and the immunization-induced forms of EAE (Bynoe et al., 2003; Bynoe and Viret, 2005). Protection was antigen-specific in that epicutaneous application of control antigens, including ovalbumin and CNS antigens other than Ac1-11 ($MOG_{35–55}$ and $PLP_{139–151}$), did not confer protection.

The epicutaneous immunization procedure also protected other mouse strains from a relapsing-remitting form of EAE. (B10.PL x SJL)F1 mice were thus protected from (Ac1-11 + CFA)-induced EAE by ECi with pure Ac1-11 (but not with pure $MOG_{35–55}$) and (SJL x PL/J)F1 mice were protected from ($PLP_{139–151}$ + CFA)-induced EAE by ECi with pure $PLP_{139–151}$ (but not with pure $MOG_{35–55}$) (Bynoe et al., 2003). More recently, we observed that ECi-induced protection also operates in the C57BL/6-MOG35–55 model of EAE (MSB, unpublished observations). The protection observed in MBP TCR Tg mice epicutaneously treated with Ac1-11 alone was mediated by antigen-specific $CD4^+$ T cells that were able to confer resistance to unmanipulated syngeneic recipients upon purification and adoptive transfer. These induced $CD4^+$ suppressor T cells inhibited, in a cell-cell contact dependent fashion, the specific proliferative response and IFN-γ production of their naïve counterparts (i.e., naïve MBP TCR Tg $CD4^+$ T cells) in vitro. ECi-induced suppressor T cells were consistently CD25 negative and did not detectably produce Th2 or Th3 response-associated major cytokines such as IL-4, IL-10, IL-13 or TGF-β upon in vitro stimulation. Administration of neutralizing antibodies to IL-4, IL-10 or TGF-β did not interfere with the protection against inducible EAE, suggesting that “Th2/3 cytokines” were not central to the protection. This was in contrast with earlier observations that IL-10 had an important role in the function of antigen specific suppressor T cells induced by repeated exposure to antigen (Wraith, 2003).

It should be emphasized that we have never noticed significant changes in the frequency of $CD25^+CD4^+$ T cells in EAE resistant mice that were epicutaneously immunized regardless of whether they were TCR transgenic or hybrid mice (relapsing-remitting model). In the same vein, $CD25^–CD4^+$ T cells purified from EAE resistant mice were always sufficient to transfer ECi-induced protection to naïve recipients. $CD4^+$ T cells isolated from disease resistant animals that were able to confer disease resistance upon transfer displayed enhanced expression of CD44 and reduced expression of CD62L relative to naïve MBP TCR Tg $CD4^+$ T cells. These CD4 T cells also did not show signs of CTLA-4 induction and were not deficient in surface expression of VLA-4 (a homing molecule crucial for migration capacity), nor did they display preferential expression of FoxP3. In vitro, these ECi-induced suppressor T cells appeared functionally inactivated; they did not proliferate or secrete IL-2 in response to specific stimulation. Exogenous IL-2 added to cultured cells did restore their proliferative capacity to some extent. Regarding the specificity of suppression, ECi-induced suppressor T cells could not suppress the proliferative response of $CD4^+$ T cells bearing a distinct antigen specificity. In a recent report, Szczepanik et al. demonstrated that ECi with the whole MBP protein could protect B10.PL mice from active EAE (Szczepanik et al., 2003). This protection was not antigen specific and was mediated by suppressor T cells that produced TGF-β (Szczepanik et al., 2005). This finding further demonstrates the importance of the experimental setting in ECi.

The context of antigen delivery was indeed a crucial factor in the generation of CD4 suppressor T cells upon epicutaneous immunization. When MBP-TCR-Tg mice were ECi with Ac1-11 emulsified in CFA prior to conventional challenge for disease induction, not only was protection aborted but the course of the disease was accelerated (Bynoe et al., 2003). Thus, efficient protection was obtained if the autoantigen was devoid of non-self determinants that are recognized by invariant receptors of the innate immune system and that confer high costimulatory potential to antigen presenting cells (Janeway, 1989; Janeway, 1992; Janeway and Medzhitov, 2002; Medzhitov and Janeway, 1997). We conclude that the two signal model of lymphocyte activation (Cohn, 1994) is adequate to describe the induction of suppressor T cells via epidermal application of pure autoantigen.

Histopathological analyses showed that there was no $CD4^+$ T cell infiltration in the brain parenchyma and no inflammatory infiltrates around CNS blood vessels within the CNS of disease resistant mice (Bynoe et al., 2003). This suggests that ECi-induced suppression most likely does not operate through the suppression of auto-aggressive T cells within the CNS itself. The suppression of auto-aggressive T cells in the CNS may be the mechanism for regulation by $CD25^+CD4^+$ T cells during the recovery from $MOG_{35–55}$-induced EAE (McGeachy et al., 2005) or in other experimental settings (Brabb et al., 2000). Thus, the protection induced by epicutaneous immunization of MBP TCR Tg mice with pure Ac1-11 peptide substantially differs from the protection induced in rats by the subcutaneous delivery of MBP emulsified in incomplete adjuvant (Swierkosz and Swanborg, 1975). This may reflect differences in species (mice versus rats), differences in antigen (peptide vs protein) or the transgenic nature of the mice used; but it could also be influenced by the route of immunization (epicutaneous vs subcutaneous).

Altogether, the results indicate that the epicutaneous application of CNS autoantigens prior to disease induction inhibits the development of EAE by interfering with the priming of naïve specific CD^4 T cells in lymphoid organs. This notion is consistent with the fact that purified CD^4 T cells from protected mice inhibit both the IFN-γ secretion by, and the proliferation of, naïve antigen specific CD^4 T cells in response to Ac1-11 exposure in vitro. In support of this interpretation, detectable serum IFN-γ level could not be induced upon immunization with Ac1-11 + CFA in mice that were previously ECi with only Ac1-11. However, this is not to say that ECi-induced suppressor T cells do not have the capacity to control autoimmune responses within the CNS. An important direction for future research that warrants investigating is whether ECi-induced suppressor T cells are capable of operating within the CNS to control EAE development.

6. Are suppressor T cells interfering with the IL-23/IL-17 pathway?

Many of the studies described above were performed prior to the discovery of the IL-23/IL-17 pathway and its pathogenic role in EAE (Hunter, 2005). The division of the Th cell lineage into Th1 and Th2 by Mosmann et al. almost twenty years ago has in large measure shaped studies in immune response designed to determine the molecular and cellular events that potentiate Th1- and Th2-cell commitment from naïve T cells (Mosmann et al., 1986). IL-12, which is produced mainly by activated dendritic cells (DCs), has been instrumental in driving the differentiation and expansion of Th1 cells (Zhang et al., 2003). Skewing the Th1 response to Th2 in EAE in the hope of a protective outcome has been a goal of many studies (Ho et al., 2005; Kumar et al., 2001). IFN-γ was one of the primary targets for immune modulation and protection in EAE (Krakowski and Owens, 1996). Later evidence indicated that IFN-γ and IL-12 were not the only factors involved in the pathogenicity of EAE, as mice deficient in these genes had more severe disease progression (Cua et al., 2003; Krakowski and Owens, 1996). Recent studies demonstrating that the IL-23/IL-17 pathway plays a prominent role in the pathogenicity of EAE (McKenzie et al., 2006) has helped to shed light on the gaps in our understanding of the role of INF-γ and IL-12 in EAE.

IL-23 is a member of the heterodimeric cytokine family and is produced by activated DCs and macrophages (Oppmann et al., 2000). IL-23 is involved in the differentiation/expansion of an effector T cell population now referred to as Th17 cells (Chen et al., 2006; McKenzie et al., 2006). Th17 cells produce the proinflammatory cytokine IL-17. IL-17 cells can potentiate autoimmune responses and EAE (Li et al., 2006; McKenzie et al., 2006). Adoptive transfer of Th-17 cells, but not Th1 cells, induces EAE in mice (Langrish et al., 2005). Neutralizing antibodies against IL-17 can ameliorate EAE (Hofstetter et al., 2005), and antibodies against IL-23 can prevent EAE induction and reverse established disease (Chen et al., 2006).

The need to re-evaluate the role of natural and induced suppressor T cells in light of the dominant role of IL-17 and IL-23 in EAE is now evident. Questions we need to address include whether epicutaneous immunization suppresses the development of Th17 cells; whether epicutaneously induced suppressor T cells can suppress IL-17 production; and whether suppression can be induced in the CNS as well as systemically (see Korn et al, this issue).

7. Future directions

7.1. From MS to EAE to MS

EAE animal models have proven invaluable in understanding distinct clinical and pathological features of MS (Gold et al., 2000; Steinman and Zamvil, 2006). A significant advantage of using mice in EAE is the ability to generate genetically-engineered mutants to study the impact of specific genes in disease. Despite numerous insights made from studies in EAE, few therapeutic applications have been successfully translated to treating MS patients. Glatiramer acetate is used for treating relapsing-remitting (R-R) MS, mitoxantrone was approved for treating secondary progressive MS, and natalizumab is approved for treating R-R-MS (Steinman and Zamvil, 2006). There is currently no cure for MS nor is there a diagnostic test that can predict whether a person will develop MS.

This is not to say we should give up studies in EAE animal models. However, maybe the time has come to take the bedside to the bench. That is, to take more advantage of available studies in humans and use the information to perform studies in animal models to gain a better understanding of the roles of various molecules in MS pathogenesis. For instance, cytokine studies in brains of MS patients by microarray gene analysis led to the discovery of IL-6 and IL-17 in MS lesions (Li et al., 2006; Lock et al., 2002). Studies in EAE demonstrated that both IL-6 and IL-17 are key players in the pathogenesis of EAE (McKenzie et al., 2006). We need to take more advantage of available data from MS patients and human samples and pursue studies in mice to gain insights into therapeutic possibilities for MS.

7.2. Emerging players in immune regulation

Several molecules were recently identified to play pivotal roles in the regulation of the immune response. Some of these include semaphorins (Sema) and their receptors, in particular, neuropilin-1 (Nrp-1) (Kikutani and Kumanogoh, 2003). Sema are axon guidance molecules originally identified by their ability to guide axons to their appropriate target cells by chemorepulsive mechanisms that induce cytoskeletal rearrangement and growth cone collapse (Moretti et al., 2006). In addition, they are involved in angiogenesis and vascularization (Moretti et al., 2006). Recent studies show that Sema are highly expressed in immune cells such as T cells, B cells, DCs and macrophages (Kikutani and Kumanogoh, 2003). Sema comprises a large family of over 30 members expressed in humans and mice (Takegahara et al., 2005). Recent studies in mice knocked out for Sema7A demonstrated that it is a negative regulator of T cell activation and function (Czopik et al., 2006). Sema4D (CD100) is constitutively expressed on T cells and promotes B cell and DC activation via its cell surface receptor, CD72 (Kikutani and Kumanogh, 2003). Similarly, Sema4A, which is expressed by DCs, activates T cells via its interaction with TIM2 (Kikutani and Kumanogoh, 2003). Sema3A, which is expressed on activated DCs and T cells can increase T cell proliferation up to 130% during DC-T cell interaction in co-cultures.

Nrp-1 is a surface receptor for the Class lll Sema, in particular, Sema3A (Gu et al., 2003). Sema3A-Nrp-1 interaction is believed to negatively regulate DC-T cell induced proliferation (Lepelletier et al., 2006). Nrp-1 is also a receptor for vascular endothelial growth factor during neural development (Gu et al., 2003). Nrp-1 is expressed on some populations of human and mouse DCs and T cells (Kikutani and Kumanogoh, 2003) and is important in the formation of

immunological synapses (Tordjman et al., 2002). Interestingly, Nrp-1 is constitutively expressed on $CD25^+CD4^+FoxP3^+$ natural suppressor T cells and is proposed to be an identifying marker for natural suppressor cells that are $FoxP3^+$ since it distinguishes Treg cells from both naive and recently activated CD4+CD25+ non-regulatory T cells (Bruder et al., 2004). Strong evidence is emerging that identifies Sema and their receptors are important modulators of the immune response. Future studies should include identifying and analyzing the role of these molecules in EAE and MS immunomodulation.

Another emerging player implicated in $CD25^+CD4^+$ $FoxP3^+$ suppressor T cell function is CD73 or 5”-ecto-nucleotidase (Kobie et al., 2006). CD73 is expressed on a subpopulation of T cells and endothelial cells (Resta et al., 1998). It catalyzes the formation of extracellular adenosine, a potent immune modulator, from AMP (Resta et al., 1998). A recent study (Kobie et al, 2006) demonstrated that natural suppressor cells express CD73. It is therefore proposed that CD73/adenosine pathway potentiates the suppressive capacity of suppressor T cells. In addition to its many other functions, adenosine exerts immunosuppressive and anti-inflammatory immune responses (Resta et al., 1998; Schaddelee et al., 2003). This pathway is potentially useful for immune response manipulation in controlling autoaggressive and proinflammatory responses. Therefore, it should be investigated as a possible therapeutic tool in EAE and MS.

Finally, an important area of study that can help dissecting immunomodulation of EAE and MS pathogenesis is chemokines and their receptors. These molecules are key in the transmigration of leukocytes across the blood brain barrier and lymphocyte trafficking into lymphoid organs (Eugenin and Berman, 2003; Mahad et al., 2006). It should be investigated whether tolerance induced in EAE models influences leukocytes influx into the CNS or the impact of lymphocyte activation and/or trafficking into lymphoid organs through modulation of chemokines and chemokine receptors.

8. Conclusions

As discussed in this review, it is likely that CD4+ suppressor T lymphocytes capable of controlling the development of EAE pathogenesis act both at very the early phases and at later time points within the CNS microenvironment. These two levels of regulation could possibly take place in a non-mutually exclusive fashion. The early control of T cells with encephalitogenic potential most likely relies on interference with efficient activation in the T cell zone of peripheral lymphoid organ, possibly through induced altered/unstable T cell-DC interaction. On the other hand, regulation of autoreactive T cells within the CNS could involve both a local secretion of immunosuppressive factors (such as IL-10 and/or TGF-β) by suppressor T cells and a repression of proinflammatory cytokine secretion (such as IFN-γ, TNF-α or IL-17) by autoreactive T cells. At the moment, it is not clear whether there exist particular subtypes of suppressor T cells that efficiently interfere with the priming of CNS antigen specific T cells systemically and other subtypes specialized in in situ immunoregulation. It is also not clear whether most suppressor T cell subsets (including induced suppressor T cells) can operate at both levels depending on the conditions. Future investigations will certainly help to determine whether or not such a dichotomy can be envisioned.

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References

Anderton SM, Wraith DC. Hierarchy in the ability of T cell epitopes to induce peripheral tolerance to antigens from myelin. Eur J Immunol 1998;28:1251–1261. [PubMed: 9565365]

Bettelli E, Das MP, Howard ED, Weiner HL, Sobel RA, Kuchroo VK. IL-10 is critical in the regulation of autoimmune encephalomyelitis as demonstrated by studies of IL-10- and IL-4-deficient and transgenic mice. J Immunol 1998;161:3299–3306. [PubMed: 9759845]

Brabb T, von Dassow P, Ordonez N, Schnabel B, Duke B, Goverman J. In situ tolerance within the central nervous system as a mechanism for preventing autoimmunity. J Exp Med 2000;192:871–880. [PubMed: 10993917]

Bruder D, Probst-Kepper M, Westendorf AM, Geffers R, Beissert S, Loser K, von Boehmer H, Buer J, Hansen W. Neuropilin-1: a surface marker of regulatory T cells. Eur J Immunol 2004;34:623–630. [PubMed: 14991591]

Burkhart C, Liu GY, Anderton SM, Metzler B, Wraith DC. Peptide-induced T cell regulation of experimental autoimmune encephalomyelitis: a role for IL-10. Int Immunol 1999;11:1625–1634. [PubMed: 10508180]

Bynoe MS, Evans JT, Viret C, Janeway CA Jr. Epicutaneous immunization with autoantigenic peptides induces T suppressor cells that prevent experimental allergic encephalomyelitis. Immunity 2003;19:317–328. [PubMed: 14499108]

Bynoe MS, Viret C. Antigen-induced suppressor T cells from the skin point of view: suppressor T cells induced through epicutaneous immunization. J Neuroimmunol 2005;167:4–12. [PubMed: 16005985]

Cabbage SE, Huseby ES, Sather BD, Brabb T, Liggitt D, Goverman J. Regulatory T cells maintain long-term tolerance to myelin basic protein by inducing a novel, dynamic state of T cell tolerance. J Immunol 2007;178:887–896. [PubMed: 17202350]

Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science 1994;265:1237–1240. [PubMed: 7520605]

Chen Y, Langrish CL, McKenzie B, Joyce-Shaikh B, Stumhofer JS, McClanahan T, Blumenschein W, Churakovsa T, Low J, Presta L, Hunter CA, Kastelein RA, Cua DJ. Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. J Clin Invest 2006;116:1317–1326. [PubMed: 16670771]

Cohn M. The wisdom of hindsight. Annu Rev Immunol 1994;12:1–62. [PubMed: 7516667]

Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, Lucian L, To W, Kwan S, Churakova T, Zurawski S, Wiekowski M, Lira SA, Gorman D, Kastelein RA, Sedgwick JD. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature 2003;421:744–748. [PubMed: 12610626]

Czopik AK, Bynoe MS, Palm N, Raine CS, Medzhitov R. Semaphorin 7A is a negative regulator of T cell responses. Immunity 2006;24:591–600. [PubMed: 16713976]

Eugenin EA, Berman JW. Chemokine-dependent mechanisms of leukocyte trafficking across a model of the blood-brain barrier. Methods 2003;29:351–361. [PubMed: 12725802]

Gold R, Hartung HP, Toyka KV. Animal models for autoimmune demyelinating disorders of the nervous system. Mol Med Today 2000;6:88–91. [PubMed: 10652482]

Goverman J, Brabb T. Rodent models of experimental allergic encephalomyelitis applied to the study of multiple sclerosis. Lab Anim Sci 1996;46:482–492. [PubMed: 8905579]

Goverman J, Brabb T, Paez A, Harrington C, von Dassow P. Initiation and regulation of CNS autoimmunity. Crit Rev Immunol 1997;17:469–480. [PubMed: 9419434]

Gu C, Rodriguez ER, Reimert DV, Shu T, Fritzsch B, Richards LJ, Kolodkin AL, Ginty DD. Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. Dev Cell 2003;5:45–57. [PubMed: 12852851]

Hardardottir F, Baron JL, Janeway CA Jr. T cells with two functional antigen-specific receptors. Proc Natl Acad Sci U S A 1995;92:354–358. [PubMed: 7530361]

Herrick CA, MacLeod H, Glusac E, Tigelaar RE, Bottomly K. Th2 responses induced by epicutaneous or inhalational protein exposure are differentially dependent on IL-4. J Clin Invest 2000;105:765–775. [PubMed: 10727445]

Ho PP, Fontoura P, Platten M, Sobel RA, DeVoss JJ, Lee LY, Kidd BA, Tomooka BH, Capers J, Agrawal A, Gupta R, Zernik J, Yee MK, Lee BJ, Garren H, Robinson WH, Steinman L. A suppressive oligodeoxynucleotide enhances the efficacy of myelin cocktail/IL-4-tolerizing DNA vaccination and treats autoimmune disease. J Immunol 2005;175:6226–6234. [PubMed: 16237121]

Hofstetter HH, Ibrahim SM, Koczan D, Kruse N, Weishaupt A, Toyka KV, Gold R. Therapeutic efficacy of IL-17 neutralization in murine experimental autoimmune encephalomyelitis. Cell Immunol 2005;237:123–130. [PubMed: 16386239]

Hori S, Carvalho TL, Demengeot J. CD25+CD4+ regulatory T cells suppress CD4+ T cell-mediated pulmonary hyperinflammation driven by Pneumocystis carinii in immunodeficient mice. Eur J Immunol 2002;32:1282–1291. [PubMed: 11981815]

Huehn J, Siegmund K, Lehmann JC, Siewert C, Haubold U, Feuerer M, Debes GF, Lauber J, Frey O, Przybylski GK, Niesner U, de la Rosa M, Schmidt CA, Brauer R, Buer J, Scheffold A, Hamann A. Developmental stage, phenotype, and migration distinguish naive- and effector/memory-like CD4+ regulatory T cells. J Exp Med 2004;199:303–313. [PubMed: 14757740]

Hunter CA. New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. Nat Rev Immunol 2005;5:521–531. [PubMed: 15999093]

Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol 1989;54(Pt 1):1–13. [PubMed: 2700931]

Janeway CA Jr. The immune system evolved to discriminate infectious nonself from noninfectious self. Immunol Today 1992;13:11–16. [PubMed: 1739426]

Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002;20:197–216. [PubMed: 11861602]

Kikutani H, Kumanogoh A. Semaphorins in interactions between T cells and antigen-presenting cells. Nat Rev Immunol 2003;3:159–167. [PubMed: 12563299]

Kim JM, Rudensky A. The role of the transcription factor Foxp3 in the development of regulatory T cells. Immunol Rev 2006;212:86–98. [PubMed: 16903908]

Kobie JJ, Shah PR, Yang L, Rebhahn JA, Fowell DJ, Mosmann TR. T regulatory and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5'-adenosine monophosphate to adenosine. J Immunol 2006;177:6780–6786. [PubMed: 17082591]

Kohm AP, Carpentier PA, Anger HA, Miller SD. Cutting edge: CD4+CD25+ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. J Immunol 2002;169:4712–4716. [PubMed: 12391178]

Krakowski M, Owens T. Interferon-gamma confers resistance to experimental allergic encephalomyelitis. Eur J Immunol 1996;26:1641–1646. [PubMed: 8766573]

Kumar V, Maglione J, Thatte J, Pederson B, Sercarz E, Ward ES. Induction of a type 1 regulatory CD4 T cell response following V beta 8.2 DNA vaccination results in immune deviation and protection from experimental autoimmune encephalomyelitis. Int Immunol 2001;13:835–841. [PubMed: 11369712]

Lafaille JJ. T-cell receptor transgenic mice in the study of autoimmune diseases. J Autoimmun 2004;22:95–106. [PubMed: 14987736]

Lafaille JJ, Nagashima K, Katsuki M, Tonegawa S. High incidence of spontaneous autoimmune encephalomyelitis in immunodeficient anti-myelin basic protein T cell receptor transgenic mice. Cell 1994;78:399–408. [PubMed: 7520367]

Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005;201:233–240. [PubMed: 15657292]

Lenz DC, Swanborg RH. Suppressor cells in demyelinating disease: a new paradigm for the new millennium. J Neuroimmunol 1999;100:53–57. [PubMed: 10695715]

Lepelletier Y, Moura IC, Hadj-Slimane R, Renand A, Fiorentino S, Baude C, Shirvan A, Barzilai A, Hermine O. Immunosuppressive role of semaphorin-3A on T cell proliferation is mediated by inhibition of actin cytoskeleton reorganization. Eur J Immunol 2006;36:1782–1793. [PubMed: 16791896]

Li B, Samanta A, Song X, Furuuchi K, Iacono KT, Kennedy S, Katsumata M, Saouaf SJ, Greene MI. FOXP3 ensembles in T-cell regulation. Immunol Rev 2006;212:99–113. [PubMed: 16903909]

Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, Langer-Gould A, Strober S, Cannella B, Allard J, Klonowski P, Austin A, Lad N, Kaminski N, Galli SJ, Oksenberg JR, Raine CS, Heller R, Steinman L. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med 2002;8:500–508. [PubMed: 11984595]

Mahad D, Callahan MK, Williams KA, Ubogu EE, Kivisakk P, Tucky B, Kidd G, Kingsbury GA, Chang A, Fox RJ, Mack M, Sniderman MB, Ravid R, Staugaitis SM, Stins MF, Ransohoff RM. Modulating CCR2 and CCL2 at the blood-brain barrier: relevance for multiple sclerosis pathogenesis. Brain 2006;129:212–223. [PubMed: 16230319]

Mathisen PM, Yu M, Johnson JM, Drazba JA, Tuohy VK. Treatment of experimental autoimmune encephalomyelitis with genetically modified memory T cells. J Exp Med 1997;186:159–164. [PubMed: 9207010]

McGeachy MJ, Stephens LA, Anderton SM. Natural recovery and protection from autoimmune encephalomyelitis: contribution of CD4+CD25+ regulatory cells within the central nervous system. J Immunol 2005;175:3025–3032. [PubMed: 16116190]

McKenzie BS, Kastelein RA, Cua DJ. Understanding the IL-23-IL-17 immune pathway. Trends Immunol 2006;27:17–23. [PubMed: 16290228]

Medzhitov R, Janeway CA Jr. Innate immunity: the virtues of a nonclonal system of recognition. Cell 1997;91:295–298. [PubMed: 9363937]

Metzler B, Wraith DC. Inhibition of experimental autoimmune encephalomyelitis by inhalation but not oral administration of the encephalitogenic peptide: influence of MHC binding affinity. Int Immunol 1993;5:1159–1165. [PubMed: 7694644]

Montero E, Nussbaum G, Kaye JF, Perez R, Lage A, Ben-Nun A, Cohen IR. Regulation of experimental autoimmune encephalomyelitis by CD4+, CD25+ and CD8+ T cells: analysis using depleting antibodies. J Autoimmun 2004;23:1–7. [PubMed: 15236747]

Moretti S, Procopio A, Boemi M, Catalano A. Neuronal semaphorins regulate a primary immune response. Curr Neurovasc Res 2006;3:295–305. [PubMed: 17109625]

Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986;136:2348–2357. [PubMed: 2419430]

Nicolson KS, O'Neill EJ, Sundstedt A, Streeter HB, Minaee S, Wraith DC. Antigen-induced IL-10+ regulatory T cells are independent of CD25+ regulatory cells for their growth, differentiation, and function. J Immunol 2006;176:5329–5337. [PubMed: 16622000]

Olivares-Villagomez D, Wang Y, Lafaille JJ. Regulatory CD4(+) T cells expressing endogenous T cell receptor chains protect myelin basic protein-specific transgenic mice from spontaneous autoimmune encephalomyelitis. J Exp Med 1998;188:1883–1894. [PubMed: 9815266]

Olivares-Villagomez D, Wensky AK, Wang Y, Lafaille JJ. Repertoire requirements of CD4+ T cells that prevent spontaneous autoimmune encephalomyelitis. J Immunol 2000;164:5499–5507. [PubMed: 10799918]

Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, Vega F, Yu N, Wang J, Singh K, Zonin F, Vaisberg E, Churakova T, Liu M, Gorman D, Wagner J, Zurawski S, Liu Y, Abrams JS, Moore KW, Rennick D, de Waal-Malefyt R, Hannum C, Bazan JF, Kastelein RA. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity 2000;13:715–725. [PubMed: 11114383]

Picca CC, Larkin J 3rd, Boesteanu A, Lerman MA, Rankin AL, Caton AJ. Role of TCR specificity in CD4+ CD25+ regulatory T-cell selection. Immunol Rev 2006;212:74–85. [PubMed: 16903907]

Resta R, Yamashita Y, Thompson LF. Ecto-enzyme and signaling functions of lymphocyte CD73. Immunol Rev 1998;161:95–109. [PubMed: 9553767]

Sakaguchi S. Naturally Arising CD4+ Regulatory T Cells for Immunologic Self-Tolerance and Negative Control of Immune Responses. Annu Rev Immunol 2004;22:531–562. [PubMed: 15032588]

Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol 2005;6:345–352. [PubMed: 15785760]

Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, Shimizu J, Takahashi T, Nomura T. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev 2006;212:8–27. [PubMed: 16903903]

Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995;155:1151–1164. [PubMed: 7636184]

Samoilova EB, Horton JL, Chen Y. Acceleration of experimental autoimmune encephalomyelitis in interleukin-10-deficient mice: roles of interleukin-10 in disease progression and recovery. Cell Immunol 1998;188:118–124. [PubMed: 9756642]

Schaddelee MP, Voorwinden HL, van Tilburg EW, Pateman TJ, Ijzerman AP, Danhof M, de Boer AG. Functional role of adenosine receptor subtypes in the regulation of blood-brain barrier permeability: possible implications for the design of synthetic adenosine derivatives. Eur J Pharm Sci 2003;19:13–22. [PubMed: 12729857]

Steinman L. Multiple sclerosis and its animal models: the role of the major histocompatibility complex and the T cell receptor repertoire. Springer Semin Immunopathol 1992;14:79–93. [PubMed: 1440199]

Steinman L, Zamvil SS. How to successfully apply animal studies in experimental allergic encephalomyelitis to research on multiple sclerosis. Ann Neurol 2006;60:12–21. [PubMed: 16802293]

Sundstedt A, O'Neill EJ, Nicolson KS, Wraith DC. Role for IL-10 in suppression mediated by peptide-induced regulatory T cells in vivo. J Immunol 2003;170:1240–1248. [PubMed: 12538682]

Swanborg RH. Antigen-induced inhibition of experimental allergic encephalomyelitis. I. Inhibition in guinea pigs injected with non-encephalitogenic modified myelin basic protein. J Immunol 1972;109:540–546. [PubMed: 4626655]

Swierkosz JE, Swanborg RH. Suppressor cell control of unresponsiveness to experimental allergic encephalomyelitis. J Immunol 1975;115:631–633. [PubMed: 50370]

Szczepanik M, Tutaj M, Bryniarski K, Dittel BN. Epicutaneously induced TGF-beta-dependent tolerance inhibits experimental autoimmune encephalomyelitis. J Neuroimmunol 2005;164:105–114. [PubMed: 15899524]

Takegahara N, Kumanogoh A, Kikutani H. Semaphorins: a new class of immunoregulatory molecules. Philos Trans R Soc Lond B Biol Sci 2005;360:1673–1680. [PubMed: 16147531]

Tordjman R, Lepelletier Y, Lemarchandel V, Cambot M, Gaulard P, Hermine O, Romeo PH. A neuronal receptor, neuropilin-1, is essential for the initiation of the primary immune response. Nat Immunol 2002;3:477–482. [PubMed: 11953749]

Van de Keere F, Tonegawa S. CD4(+) T cells prevent spontaneous experimental autoimmune encephalomyelitis in anti-myelin basic protein T cell receptor transgenic mice. J Exp Med 1998;188:1875–1882. [PubMed: 9815265]

Wang LF, Lin JY, Hsieh KH, Lin RH. Epicutaneous exposure of protein antigen induces a predominant Th2-like response with high IgE production in mice. J Immunol 1996;156:4077–4082. [PubMed: 8666772]

Wang LF, Sun CC, Wu JT, Lin RH. Epicutaneous administration of hapten through patch application augments TH2 responses which can downregulate the elicitation of murine contact hypersensitivity. Clin Exp Allergy 1999;29:271–279. [PubMed: 10051733]

Weiner HL. Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells. Immunol Rev 2001a;182:207–214. [PubMed: 11722636]

Weiner HL. Oral tolerance: immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. Microbes Infect 2001b;3:947–954. [PubMed: 11564443]

Wraith DC. Role of interleukin-10 in the induction and function of natural and antigen-induced regulatory T cells. J Autoimmun 2003;20:273–275. [PubMed: 12791311]

Wu HY, Weiner HL. Oral tolerance. Immunol Res 2003;28:265–284. [PubMed: 14713719]

Yu P, Gregg RK, Bell JJ, Ellis JS, Divekar R, Lee HH, Jain R, Waldner H, Hardaway JC, Collins M, Kuchroo VK, Zaghouani H. Specific T regulatory cells display broad suppressive functions against

experimental allergic encephalomyelitis upon activation with cognate antigen. J Immunol 2005;174:6772–6780. [PubMed: 15905518]

Zhang GX, Gran B, Yu S, Li J, Siglienti I, Chen X, Kamoun M, Rostami A. Induction of experimental autoimmune encephalomyelitis in IL-12 receptor-beta 2-deficient mice: IL-12 responsiveness is not required in the pathogenesis of inflammatory demyelination in the central nervous system. J Immunol 2003;170:2153–2160. [PubMed: 12574388]

Zhang X, Koldzic DN, Izikson L, Reddy J, Nazareno RF, Sakaguchi S, Kuchroo VK, Weiner HL. IL-10 is involved in the suppression of experimental autoimmune encephalomyelitis by CD25+CD4+ regulatory T cells. Int Immunol 2004;16:249–256. [PubMed: 14734610]