8-1-2015

Mechanisms of immunological tolerance in central nervous system inflammatory demyelination.

Elisabeth R. Mari
Thomas Jefferson University

Jason N. Moore
Thomas Jefferson University

Guang-Xian Zhang
Thomas Jefferson University

A. M. Rostami
Thomas Jefferson University

HHS Public Access

Author manuscript

Clin Exp Neuroimmunol. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Clin Exp Neuroimmunol. 2015 August 1; 6(3): 264–274. doi:10.1111/cen3.12196.

Mechanisms of immunological tolerance in central nervous system inflammatory demyelination

Elisabeth R. Mari, **Jason N. Moore**, **Guang-Xian Zhang**, and **Abdolmohamad Rostami**

Department of Neurology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

Abstract

Multiple sclerosis is a complex autoimmune disease of the central nervous system that results in a disruption of the balance between pro-inflammatory and anti-inflammatory signals in the immune system. Given that central nervous system inflammation can be suppressed by various immunological tolerance mechanisms, immune tolerance has become a focus of research in the attempt to induce long-lasting immune suppression of pathogenic T cells. Mechanisms underlying this tolerance induction include induction of regulatory T cell populations, anergy and the induction of tolerogenic antigen-presenting cells. The intravenous administration of encephalitogenic peptides has been shown to suppress experimental autoimmune encephalomyelitis and induce tolerance by promoting the generation of regulatory T cells and inducing apoptosis of pathogenic T cells. Safe and effective methods of inducing long-lasting immune tolerance are essential for the treatment of multiple sclerosis. By exploring tolerogenic mechanisms, new strategies can be devised to strengthen the regulatory, anti-inflammatory cell populations thereby weakening the pathogenic, pro-inflammatory cell populations.

Keywords

interleukin-10; interleukin-27; immune tolerance; multiple sclerosis; regulatory T cells

Introduction

Multiple sclerosis (MS) is a chronic, autoimmune disease of the central nervous system (CNS). It is primarily characterized by inflammatory damage to the myelin sheath and axonal degeneration, leading to neurological disability.[1] Normal immune function in the CNS is characterized by a combination of pro- and anti-inflammatory signals. These signals become dysregulated by increased pro-inflammatory stimulus in MS, leading to local tissue damage and the formation of lesions. The underlying immune process is also thought to be heavily reliant on $CD4^+$ T cells.[2] MS pathology has been extensively studied using the animal model of experimental autoimmune encephalomyelitis (EAE). In this model, EAE can be actively induced with an injection of a myelin protein and complete Freund's adjuvant, resulting in the production of pathogenic, myelin-specific T cells. These cells,

Correspondence: Abdolmohamad Rostami, PhD, MD, Department of Neurology Thomas Jefferson University, 901 Walnut Street, Suite 400, Philadelphia, PA, 19107, USA, Tel: 215 955 8100, Fax: 215 955 1390, A.M.Rostami@jefferson.edu.

The authors declare no conflict of interest.

which have developed in the peripheral lymphoid organs, infiltrate the CNS. In the CNS, infiltrating autoreactive T helper (Th $CD4^+$) cells encounter their cognate antigens presented by antigen-presenting cells (APC).[3–11] The result is reactivation of $CD4^+$ T cells, which, in turn, activate APC by cell–cell contact and pro-inflammatory cytokines. These pro-inflammatory signals recruit immune cells, such as $CD8^+$ T cells, macrophages/dendritic cells (DC), mast cells and activated microglia, which cause local tissue damage.[7–10]

It has been known for decades that inflammation in the CNS can be suppressed by various immunological tolerance mechanisms. Our laboratory, along with others, has established methods to elucidate these mechanisms, which include induction of regulatory T populations, immune deviation and induction of APC. These APC produce immunoregulatory cytokines, such as interleukin (IL)-10, transforming growth factor-β (TGF-β) and IL-27.[9, 10] Immune tolerance can be induced in EAE by the administration of encephalotogenic antigens in a variety of tolerogenic forms and by various routes.[12–14] In the present article, we review the key mechanisms underlying immune tolerance in MS and EAE, and their impact on future therapeutic intervention in these diseases.

Pathogenic Th1 and Th17 cells

A significant amount of MS research has been focused on $CD4^+$ (Th) cells based on the hypothesis that they play a central role in CNS autoimmunity. In EAE animals, the immune responses that develop after immunization are largely governed by interferon-γ $(IFN\text{-}\gamma)^+$ Th1 cells, which are the most abundant $CD4^+$ T cells observed in the CNS of animals after immunization with myelin peptides.[3, 15] Additionally, Th1, but not Th2 myelin-specific cells, were able to induce EAE when adoptively transferred into recipient mice.[16, 17] Furthermore, in MS relapse, elevated levels of Th1 cytokines have been observed in MS patients when compared with healthy controls, whereas Th2 cytokines are present during remission in MS patients.

EAE has been considered a typical Th1-mediated disease, but recent data show that Th17 cells play an important role in the pathogenesis of EAE.[18, 19] Studies by Harrington *et al.* and Wang *et al.* first described this lineage of Th cells that express the cytokine IL-17A and whose development is driven by IL-23.[7,20] In CNS autoimmunity, immunization with myelin antigens induces the development of Th17 cells, and these myelin-specific cells traffic to the CNS, where they secrete IL-17A. IL-17A attracts various immune cells and, in particular, myeloid cells into the CNS, thus starting and propagating the inflammatory cascade.[10, 21–25]

There is evidence from MS patients supporting the pathogenic role of Th17 cells in disease development. These findings come from Tzartos *et al.*, who found a relative increase in Th17 cells in the lesions of active MS patients when compared with healthy controls.[26] In addition, Durelli *et al.* found a sevenfold increase in the fraction of Th17 cells in untreated patients with active MS compared with those with inactive MS and healthy controls.[27] In contrast, the Th1 cell population was not always found to increase. They also found that treatment with interferon-β led to apoptosis in the Th17, but not the Th1 cell population, a finding that has been confirmed by other research groups.[28] Suppression of these highly

pathogenic T cell subsets is crucial for long-lasting immune tolerance and attenuation of disease. Recent studies have shown that the newer MS agents, dimethyl fumarate and fingolimod, also decrease the population of Th17 cells.[29, 30] However, the immune mechanisms used by these drugs to maintain suppression of pathogenic Th cell subsets have not yet been elucidated.

Regulatory T cells

Immune tolerance has increasingly become a focus in MS research. In EAE, the immune system can become tolerized to myelin-specific antigens and anti-inflammatory mediators that inhibit pro-inflammatory signals, reducing inflammatory stress. A key component in this system is regulatory T cells.

Regulatory $CD4^+CD25^+$ T cells (Tregs) are part of the $CD4^+CD25^+$ effector T cell population. They are distinguished from these cells by the expression of the forkhead/winged helix family transcription factor forkhead box P3 (FoxP3), FR4 and constitutive expression of CTLA-4 (CD152).[31–37] In mice, the lymphocyte activation gene-3 (LAG-3) is also constitutively expressed on the surface of Treg cells.[38] In addition to the thymic-derived or “natural” (nTregs), Tregs can also be induced (iTregs) in the periphery. iTregs can be $FoxP3^+$, but, under a variety of conditions, they develop in the periphery from conventional $CD4^+$ T cells after antigen stimulation.[39] T helper 3 (Th3) cells are a population of iTreg cells that produce larger amounts of TGF-β and occur primarily after exposure to antigen through the oral route.[40] T regulatory 1 (Tr1) cells are a subset of iTregs, and are similar to nTregs in that they are both anergic *in vitro* and express CTLA-4. Induction of these cells can occur through stimulation by immature DC, in the presence of IL-10.[41]

Tregs are able to exert their suppressive effects on immune responses by limiting activation, proliferation and survival of various immune cells. These functions are exerted through direct cell–cell contact and cytokine production, and the depletion of Tregs in mice leads to autoimmunity.[42, 43] This autoimmunity can be prevented by the administration of $CD4^+CD25^+$ T cells to newborn mice.[43] In humans, a mutation in the FoxP3 gene causes immune dysregulation, polyendocrinopathy and enteropathy, and X-linked syndrome, which results in the early onset of one or more autoimmune diseases.[44, 45]

$FoxP3^+$ Tregs in MS/EAE

A role for Tregs in the modulation of neuroinflammatory responses and maintenance of self-tolerance is supported by both animal and human studies. In EAE, enhanced disease severity and mortality were observed when Tregs were depleted after treatment with anti-CD25 antibodies.[46] Adoptive transfer of Tregs into mice immunized with myelin oligodendrocyte glycoprotein (MOG) 35–55 conferred significant protection from EAE induction.[47] In addition, the authors observed increased frequencies of $MOG_{35–55}$-specific Th2 cells and decreased CNS infiltration.[48] In a recent report, Joller *et al.* identified a subpopulation of Tregs that express a co-inhibitory molecule, TIGIT, which on ligation induced expression of the effector molecule fibrinogen-like protein 2, and induced Treg-cell mediated suppression of Th1 and Th17 cells.[49] The aforementioned studies show that Tregs inhibit priming and

expansion of pathogenic Th1 and Th17 cells in the peripheral lymphoid organs, thus directly contributing to the maintenance of self-tolerance.

Studies of the peripheral blood from MS patients showed an important role for Tregs in disease pathogenesis. The Hafler group reported defects in the function, but not the frequency, of $CD4^+CD25^{high}$ Treg populations in RR-MS patients.[50] Using a coculture assay with circulating $CD4^+CD25^{high}$ Tregs from relapsing–remitting MS (RR-MS) patients, they showed that these cells had a reduced capacity to suppress polyclonally activated $CD4^+CD25^-$ conventional T cells.[50] Hafler's group and others have shown that this functional alteration in Treg suppression in MS patients is due to a reduced expression of FoxP3 at both the mRNA and protein level.[51, 52] In addition, the Treg population in MS patients tends to have a lower proportion of newly generated cells from the thymus, as evidenced by a lower proportion of $CD31^+$ and $CD45RA^+$ cells, and a higher proportion of older, memory cells with the $CD45RO^+$ phenotype.[53, 54]

Furthermore, these dysfunctional Tregs in MS patients cannot carry out the same suppressive functions as those in healthy individuals. Extracellular adenosine triphosphate (ATP) acts as a pro-inflammatory cytokine, which can induce the secretion of IL-1β and IL-23, and lead to increased production of pathogenic Th17 cells.[55, 56] CD39 acts as an ectoenzyme and hydrolyzes ATP to adenosine monophosphate. Additionally, $CD39^+$ Tregs have been shown to catabolize extracellular ATP, resulting in decreased secretion of these inflammatory cytokines and a smaller Th17 population.[57] MS patients have decreased amounts of $CD39^+$ Tregs when compared with healthy controls.[55, 58] Recently, it has been shown that fingolimod is able to increase the proportion of $FoxP3^+$ $CD39^+$ Tregs within the $CD4^+$ T cell population in patients with RR-MS.[59]

These alterations in Treg function might also be explained by a production of pro-inflammatory cytokines. Studies by Dominquez-Villar *et al.* compared the Tregs in patients with untreated RR-MS with healthy controls, and found an increased proportion of IFN-γ producing Tregs in the patients, making these cells similar to pathogenic Th1 cells.[60] These cells still expressed FoxP3, while also expressing the transcription factor TBET and CXCR3, both of which are usually expressed on Th1 cells.[60] These Th1-like cells were also found to be less suppressive *ex vivo.* Many MS therapies have been shown to target the Treg population and to reverse the suppressive defects observed.[61–63] Interferon β-1a and glatiramer, in particular, have been shown to expand the native Treg population *in vivo* and to partially restore their suppressive function.[64, 65] The Hafler group also showed that treatment with IFN-β1a reduced the number of Tregs with the Th1-like phenotype.[60]

Despite the overwhelming evidence of the role of Tregs in the maintenance of self-tolerance in MS, their function in the CNS remains unclear. It has been previously shown that microglia can recruit Tregs into the CNS through the production of CCL22, which interacts with CCR4, expressed on the surface of Tregs.[66, 67] In both active and passive models of EAE, an accumulation of Tregs in the CNS correlates with disease recovery, and Tregs from these animals suppressed MOG-specific T cell responses by limiting IFN-γ production.[68, 69] Studies by Korn *et al.* reported that Tregs accumulated in the inflamed CNS, but lacked suppressive capabilities because of the presence of IL-6 and TNF-α.[70] Importantly, IL-6

signaling inhibits the conversion of conventional T cells into FoxP3$^+$ Tregs *in vivo*.[70, 71] These results suggest that Tregs have a role in suppressing inflammation in the CNS, which is dependent on the local inflammatory setting and the effector T cell populations.

Type 1 regulatory T cells in MS and EAE

The highly immunosuppressive subset of iTregs, Tr1 cells, is believed to play a significant role in the maintenance of immunological tolerance.[72, 73] Tr1 cells are a subset of regulatory cells in which FoxP3 and CD25 are not expressed. These cells can be generated in the absence of nTregs, suggesting that Tr1 cells might be developmentally distinct.[74, 75] These cells are characterized by their secretion of high amounts of IL-10 and the expression of the newly defined cell surface markers, CD49b and LAG-3, which can be found on both human and murine cells.[76] Tr1 cells have a low proliferative capability, but can expand in the presence of IL-2 and IL-15 because of high expression of these receptors after activation.[77] The main mechanisms of Tr1-mediated suppression are secretion of a high level of IL-10 and the killing of APC by granzyme B.[78, 79]

Recently, the immunomodulatory cytokine, IL-27, has been identified as a differentiation factor for the generation of both human and murine IL-10-producing Tr1 cells.[80–82] T cell activation in the presence of IL-27 induces the transcription factors c-Maf and the aryl hydrocarbon receptor. Activation of these transcription factors is crucial for the differentiation and secretion of IL-10 from developing Tr1 cells.[83–85] Studies by Gandhi *et al.* have shown that aryl hydrocarbon receptor plays an important role in Tr1 differentiation in humans, and suggests that this could be a possible mechanism to target the generation of iTregs in autoimmune disease.[86] Tr1 cells have also been shown to play a suppressive role in MS and EAE. In EAE, the transfer of *in vitro* generated OVA-specific Tr1 cells prevented the development of neurological symptoms when OVA peptide was injected intracranially.[75] In EAE, the *in vivo* induction of Tr1 cells was achieved using soluble myelin basic protein (MBP) p87–99, which reversed ongoing disease in rats immunized with MBP.[87]

In MS patients, Astier *et al.* showed that Tr1 cells isolated from these patients had impaired IL-10 production when compared with healthy controls. Although Tr1 cells were impaired, the levels of IFN-γ production remained consistent.[88, 89] Additionally, Martinez-Forero *et al.* found that IL-10-mediated suppressive effects of Tr1 cells were reduced in *ex vivo* samples isolated from MS patients.[90] Taken together, these studies suggest that Tr1 cells might play a protective role in MS.[88]

DCs and IL-27 in immune tolerance induction

DC prime T cells for inflammatory responses, but these cells can have a dual role and also promote the development of tolerance. Evidence supporting this dual role comes from studies in which DC were completely ablated, which resulted in fatal autoimmunity.[91] To further support their tolerogenic role, Yamazaki *et al.* showed that DC can induce expansion of the CD25$^+$ CD4$^+$ T cell population;[92] in addition, Darrassee-Jeze *et al.* showed that a decrease in DC leads to a decrease in Tregs.[93] iDC, which have low expression of major

histocompatibility class II and costimulatory molecules, are able to induce tolerance through T cell anergy.[93]

One of the main mechanisms by which DC contribute to tolerance induction is through the induction of Tregs. This process is dependent on TGF-β, and absence of this cytokine has been shown to reduce the ability of DC to stimulate a pathogenic response from T cells.[94] In EAE, the absence of TGF-β resulted in more severe EAE in mice. In addition, DC were able to stimulate the expansion of $Foxp3^+$ Tregs in the presence of TGF-β and retinoic acid. When these TGF-β-induced Tregs were expanded and separated, they were able to suppress EAE.[95]

DC also exert some of their tolerogenic effect through the immunomodulatory cytokine, IL-27, a member of the IL-12 family of cytokines. IL-27 is composed of EBV-induced molecule 3 (EBI3), an IL-12p40 homolog, and p28, an IL-12p35 homolog, which non-covalently associate with EBI3, with its main sources of IL-27 being DC and macrophages.[96] The IL-27 receptor is composed of WSX1 and gp130 (a part of the IL-6 receptor complex)[97,98], and signaling through IL-27 receptors results in phosphorylation of Jak and Stat proteins, including Jak1, Jak2, Tyk2, Stat1, Stat3, Stat4 and Stat5.[99–102]

Initially, IL-27 was thought only to promote Th1 cell differentiation[99], but studies have shown that this cytokine plays a crucial role in limiting Th17 cell differentiation by suppressing RORγt, a key transcription factor for Th17 cells.[103] Mice deficient in IL-27 signaling, either EBI3 or WSX, have increased IL-17 and are more susceptible to EAE.[103, 104] Data from our laboratory show that IL-27 has a suppressive effect on encephalitogenic Th17 cells and the effector phase of EAE.[105] The suppression of Th17 cells and EAE by IL-27 is associated with IL-27-induced production of IL-10 in T cells, including Th1 but not Th17 cells.[81] IL-27 induction of the IL-10-producing immunosuppressive Tr1 cell subset is STAT1- and STAT3-dependent, and induces the transcription factor, c-maf, which can in turn activate IL-21 production by T cells.[99] Along with IL-21, IL-27 can also induce upregulation of IL-21 receptor, which can act in an autocrine manner to promote Tr1 cell growth and differentiation.[82] Xu *et al.* showed that in the absence of IL-21 signaling in T cells, IL-27 driven generation of Tr1 cells and IL-10 cytokine production is inhibited.[106] However, IL-27 alone can increase IL-10 production for only a limited period of time. Studies by Awasthi *et al.* showed that simultaneous stimulation with IL-27 and TGF-β caused a long-lasting production of IL-10 by T cells.[80]

Because of the significant immunomodulatory properties of IL-27, it is thought to have a therapeutic potential in MS. Fitzgerald *et al.* showed that exogenous IL-27 could reduce the severity of EAE when delivered by subcutaneous osmotic pump. IL-27 reduced infiltration of Th17 cells into the CNS and inhibited IL-17A production by myelin-specific T cells.[105] A recent study by Mascanfioni *et al.* showed that IL-27 signaling in DC upregulates CD39 expression and limits EAE severity by reducing the extracellular levels of ATP. They showed that these IL-27-conditioned DC can suppress EAE when administered i.v. in a chronic EAE model.[107] These studies show the variety of potential therapeutic uses for IL-27.

Mediators of tolerance

I.v. tolerance

In EAE, antigen-specific tolerance can be achieved by i.v. administration of encephalitogenic antigens.[108] Treatment of $CD4^+$ T cells with antigen has been shown to reduce the production of cytokines and decrease the extent of their antigen-specific proliferation.[108, 109] This is augmented by multiple treatments with antigen, especially for memory T cells, which might not be fully suppressed on interaction with only one round of antigen.[110] Induction of i.v. tolerance is associated with the disappearance of transgene-positive T cells from peripheral lymphoid tissues.[110] In an adoptive transfer model of EAE, multiple i.v. injections of MBP to recipient mice after transfer of MBP-specific TCR transgenic T cells prevented disease.[111] Administering antigen i.v. is more effective in inducing tolerance after disease onset when compared with mucosal routes.[112, 113]

Antigen-specific tolerance is produced by a multifactorial mechanism. Mechanisms of tolerance induction include clonal deletion, anergy, induction of regulatory T cell populations and upregulation of CTLA-4.[12–14,108,114,115] Induction of i.v. tolerance is associated with the disappearance of transgene-positive T cells from peripheral lymphoid tissues. Our group has shown that i.v. injection of the myelin autoantigen suppressed antigen-specific Th1 and Th17 responses, and induced nTregs and IL-10-producing Tr1 cells (Figure 1).[116,117] Li *et al.* discovered that i.v. tolerized mice had increased proportions of tolerogenic DC in their spleens and CNS. When these DC were transferred into recipient mice with ongoing EAE, they were able to suppress disease by inhibiting $MOG_{35–55}$-specific T cell proliferation, and by inducing nTregs and the regulatory cytokines, IL-10 and IL-27.[117]

I.v. tolerance is currently being explored as a potential therapeutic for the treatment of MS. Common methods of introducing antigen i.v. include using a soluble peptide or linking the peptide to splenocytes or microparticles. However, the use of soluble peptide i.v. carries the risk of an anaphylactic reaction, which has been shown to develop in several strains of mice.[118] The administration of antigen-coupled splenocytes or microparticles is not known to cause these types of reaction and thus provides a safer method of peptide delivery.

Antigens of interest can be coupled to splenocytes using 1-ethyl-3-(3-dimethylaminopropyl)-carbodimide.[119] Infusion of these antigen-linked splenocyctes has been shown to induce the rapid production of IL-10 and TGF-β, leading to anergy of pathogenic T cells and the induction of Tregs.[119, 120] It is thought that antigen-linked splenocyctes undergo rapid apoptosis after i.v. infusion, and this apoptotic debris is taken up by marginal zone macrophages, leading to the production of IL-10.[121] Additionally, the antigen-linked splenocyctes might interact directly with autoreactive T cells, leading to anergy.

Recently, Lutterotti *et al.* examined these principles of tolerance in a translational phase 1 trial in human subjects.[122] They studied nine patients with RR-MS or secondary progressive MS who were reactive to one of seven myelin proteins. For this trial, they administered a one-dose cocktail of myelin proteins, chemically coupled to the patients' own peripheral

blood mononuclear cells. They studied escalating doses from 1×10^3 and 3×10^9 peripheral blood mononuclear cells, and patients were observed over a 3-month period post-infusion. The primary purpose of the study was to establish feasibility, safety and tolerability of this regimen, which, overall, proved to be feasible, with generally mild side-effects. At higher doses, the study authors noted a decrease in T cell response to the myelin proteins indicative of successful tolerization.

The production and storage of leukocytes is, however, expensive and problematic. In a recent study, Getts *et al.* developed a new method of i.v. tolerance induction by covalently coupling myelin-specific antigens to biodegradable microparticles.[119] These microparticles were made of a relatively inexpensive polystyrene or poly(lactide-*co*-glycolide) measuring 500 nm in diameter. The antigen-linked particles were then i.v. injected into mice suffering from relapsing EAE, leading to significant amelioration of clinical disease.[119] These antigen-coupled microparticles suppressed Th1 and Th17 cell proliferation, and the primary mechanism of tolerance induction observed was T cell anergy. Hunter *et al.* continued this line of research, and found that i.v. administration of antigen-linked nanoparticles led to amelioration of disease.[123] These studies highlight the potential therapeutic uses of antigen-specific i.v. tolerance for the treatment of MS.

Galectin-1

The role of lectin-binding proteins and tolerance induction in autoimmunity is an emerging field. It has been observed that galectin-1, a member of the β-galactosidase binding protein family, plays an important immunoregulatory role in EAE.[124, 125] Galectin-1 is synthesized and secreted by nTregs and iTregs, as well as by activated B cells and T cells, inflammatory macrophages, and decidual natural killer cells, and it is upregulated on TCR activation.[126, 127] Mice deficient in galectin-1 have augmented Th1 and Th17 responses, and are more susceptible to autoimmune diseases and immune-mediated fetal rejection when compared with wild-type mice.[125]

Ilarregui *et al.* showed that galectin-1 significantly increased the development of Tr1 cells *in vitro* through the generation of tolerogenic DC.[124] These tolerogenic DC produce IL-27, which acts on T cells and promotes their IL-10 production. Additionally, treatment of naïve T cells with recombinant mouse galectin-1 induced Tr1 cells, which were able to suppress Th1- and Th17-mediated inflammation.[124] Galectin-1 is upregulated in Treg cells on TCR activation, and blockade of galectin-1 binding reduces inhibitory activity of human and mouse Tregs.[128–130]

Conclusions

MS is a complex disease with the involvement of multiple pathways that can lead to CNS inflammation. Studies on the primary pathogenic (Th1/Th17) and the tolerogenic (Treg/Tr1/IL-27-producing) mechanisms used to combat these pathogenic responses have considerably enhanced our knowledge of disease pathogenesis. By continuing to study their underlying mechanisms, we can further understand the complex balance these cells must maintain to preserve homeostasis in the healthy individual.

There is evidence that the currently established medications for MS work, at least in part, by using these tolerogenic mechanisms. Recent findings have shown the potential therapeutic uses of tolerogenic cells and antigen-coupled nanoparticles to treat EAE and MS. By continuing to explore this field of research, we should be able to develop improved therapeutic interventions that better target the underlying dysfunction, potentially resulting in more effective therapy and with fewer adverse events. In short, induction of these tolerogenic immune cell subsets can help to direct the future of MS therapeutics.

Acknowledgments

This work was supported by grants from the NIH/NINDS 2R01NS048435. We thank K. Regan for editorial assistance.

References

1. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. The New England journal of medicine. 2000 Sep 28; 343(13):938–52. [PubMed: 11006371]
2. Rostami A, Ciric B. Role of Th17 cells in the pathogenesis of CNS inflammatory demyelination. Journal of the neurological sciences. 2013 Oct 15; 333(1–2):76–87. [PubMed: 23578791]
3. Ando DG, Clayton J, Kono D, Urban JL, Sercarz EE. Encephalitogenic T cells in the B10.PL model of experimental allergic encephalomyelitis (EAE) are of the Th-1 lymphokine subtype. Cellular immunology. 1989 Nov; 124(1):132–43. [PubMed: 2478300]
4. Yang Y, Weiner J, Liu Y, et al. T-bet is essential for encephalitogenicity of both Th1 and Th17 cells. The Journal of experimental medicine. 2009 Jul 6; 206(7):1549–64. [PubMed: 19546248]
5. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual review of immunology. 1989; 7:145–73.
6. Segal BM. Experimental autoimmune encephalomyelitis: cytokines, effector T cells, and antigen-presenting cells in a prototypical Th1-mediated autoimmune disease. Current allergy and asthma reports. 2003 Jan; 3(1):86–93. [PubMed: 12543000]
7. Langrish CL, Chen Y, Blumenschein WM, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. The Journal of experimental medicine. 2005 Jan 17; 201(2): 233–40. [PubMed: 15657292]
8. El-Behi M, Ciric B, Dai H, et al. The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. Nature immunology. 2011 Jun; 12(6):568–75. [PubMed: 21516111]
9. Secor VH, Secor WE, Gutekunst CA, Brown MA. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. The Journal of experimental medicine. 2000 Mar 6; 191(5):813–22. [PubMed: 10704463]
10. Kroenke MA, Carlson TJ, Andjelkovic AV, Segal BM. IL-12- and IL-23-modulated T cells induce distinct types of EAE based on histology, CNS chemokine profile, and response to cytokine inhibition. The Journal of experimental medicine. 2008 Jul 7; 205(7):1535–41. [PubMed: 18573909]
11. Gregory GD, Raju SS, Winandy S, Brown MA. Mast cell IL-4 expression is regulated by Ikaros and influences encephalitogenic Th1 responses in EAE. The Journal of clinical investigation. 2006 May; 116(5):1327–36. [PubMed: 16628252]
12. Miller A, Zhang ZJ, Sobel RA, al-Sabbagh A, Weiner HL. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. VI. Suppression of adoptively transferred disease and differential effects of oral vs. intravenous tolerization. Journal of neuroimmunology. 1993 Jul; 46(1–2):73–82. [PubMed: 7689596]
13. Liblau RS, Tisch R, Shokat K, et al. Intravenous injection of soluble antigen induces thymic and peripheral T-cells apoptosis. Proceedings of the National Academy of Sciences of the United States of America. 1996 Apr 2; 93(7):3031–6. [PubMed: 8610163]

14. Zhang GX, Yu S, Calida D, et al. Loss of the surface antigen 3G11 characterizes a distinct population of anergic/regulatory T cells in experimental autoimmune encephalomyelitis. Journal of immunology. 2006 Mar 15; 176(6):3366–73.
15. Voskuhl RR, McFarlin DE, Tranquill LR, et al. A novel candidate autoantigen in a multiplex family with multiple sclerosis: prevalence of T-lymphocytes specific for an MBP epitope unique to myelination. Journal of neuroimmunology. 1993 Jul; 46(1–2):137–44. [PubMed: 7689584]
16. van der Veen RC, Stohlman SA. Encephalitogenic Th1 cells are inhibited by Th2 cells with related peptide specificity: relative roles of interleukin (IL)-4 and IL-10. Journal of neuroimmunology. 1993 Nov-Dec;48(2):213–20. [PubMed: 8227319]
17. Markiewicz K, Cholewa M, Luciak M. Influence of tobacco smoking on serum free fatty acid, triglyceride and glucose levels during physical training and post-exertional restitution. Acta medica Academiae Scientiarum Hungaricae. 1978; 35(3–4):225–32. [PubMed: 756111]
18. Zhang GX, Gran B, Yu S, et al. 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. Journal of immunology. 2003 Feb 15; 170(4):2153–60.
19. Leonard JP, Waldburger KE, Goldman SJ. Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. The Journal of experimental medicine. 1995 Jan 1; 181(1):381–6. [PubMed: 7528773]
20. Harrington LE, Hatton RD, Mangan PR, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nature immunology. 2005 Nov; 6(11):1123–32. [PubMed: 16200070]
21. Komiyama Y, Nakae S, Matsuki T, et al. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. Journal of immunology. 2006 Jul 1; 177(1):566–73.
22. Haak S, Croxford AL, Kreymborg K, et al. IL-17A and IL-17F do not contribute vitally to autoimmune neuro-inflammation in mice. The Journal of clinical investigation. 2009 Jan; 119(1): 61–9. [PubMed: 19075395]
23. Tigno-Aranjuez JT, Jaini R, Tuohy VK, Lehmann PV, Tary-Lehmann M. Encephalitogenicity of complete Freund's adjuvant relative to CpG is linked to induction of Th17 cells. Journal of immunology. 2009 Nov 1; 183(9):5654–61.
24. Chen Z, Laurence A, Kanno Y, et al. Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. Proceedings of the National Academy of Sciences of the United States of America. 2006 May 23; 103(21):8137–42. [PubMed: 16698929]
25. Uyttenhove C, Van Snick J. Development of an anti-IL-17A auto-vaccine that prevents experimental auto-immune encephalomyelitis. European journal of immunology. 2006 Nov; 36(11):2868–74. [PubMed: 17048276]
26. Tzartos JS, Friese MA, Craner MJ, et al. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. The American journal of pathology. 2008 Jan; 172(1):146–55. [PubMed: 18156204]
27. Durelli L, Conti L, Clerico M, et al. T-helper 17 cells expand in multiple sclerosis and are inhibited by interferon-beta. Annals of neurology. 2009 May; 65(5):499–509. [PubMed: 19475668]
28. Ramgolam VS, Sha Y, Jin J, Zhang X, Markovic-Plese S. IFN-beta inhibits human Th17 cell differentiation. Journal of immunology. 2009 Oct 15; 183(8):5418–27.
29. Mehling M, Lindberg R, Raulf F, et al. Th17 central memory T cells are reduced by FTY720 in patients with multiple sclerosis. Neurology. 2010 Aug 3; 75(5):403–10. [PubMed: 20592255]
30. Peng H, Guerau-de-Arellano M, Mehta VB, et al. Dimethyl fumarate inhibits dendritic cell maturation via nuclear factor kappaB (NF-kappaB) and extracellular signal-regulated kinase 1 and 2 (ERK1/2) and mitogen stress-activated kinase 1 (MSK1) signaling. The Journal of biological chemistry. 2012 Aug 10; 287(33):28017–26. [PubMed: 22733812]
31. 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 Aug 1; 155(3):1151–64. [PubMed: 7636184]

32. Kuniyasu Y, Takahashi T, Itoh M, Shimizu J, Toda G, Sakaguchi S. Naturally anergic and suppressive CD25(+)CD4(+) T cells as a functionally and phenotypically distinct immunoregulatory T cell subpopulation. International immunology. 2000 Aug; 12(8):1145–55. [PubMed: 10917889]
33. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nature immunology. 2003 Apr; 4(4):330–6. [PubMed: 12612578]
34. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003 Feb 14; 299(5609):1057–61. [PubMed: 12522256]
35. Yamaguchi T, Hirota K, Nagahama K, et al. Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor. Immunity. 2007 Jul; 27(1):145–59. [PubMed: 17613255]
36. Takahashi T, Tagami T, Yamazaki S, et al. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. The Journal of experimental medicine. 2000 Jul 17; 192(2):303–10. [PubMed: 10899917]
37. Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. The Journal of experimental medicine. 2000 Jul 17; 192(2):295–302. [PubMed: 10899916]
38. Huang CT, Workman CJ, Flies D, et al. Role of LAG-3 in regulatory T cells. Immunity. 2004 Oct; 21(4):503–13. [PubMed: 15485628]
39. Stephens LA, Mason D. CD25 is a marker for CD4+ thymocytes that prevent autoimmune diabetes in rats, but peripheral T cells with this function are found in both CD25+ and CD25− subpopulations. Journal of immunology. 2000 Sep 15; 165(6):3105–10.
40. Faria AM, Weiner HL. Oral tolerance: mechanisms and therapeutic applications. Advances in immunology. 1999; 73:153–264. [PubMed: 10399007]
41. Sundstedt A, O'Neill EJ, Nicolson KS, Wraith DC. Role for IL-10 in suppression mediated by peptide-induced regulatory T cells in vivo. Journal of immunology. 2003 Feb 1; 170(3):1240–8.
42. Russell WL, Russell LB, Gower JS. Exceptional Inheritance of a Sex-Linked Gene in the Mouse Explained on the Basis That the X/O Sex-Chromosome Constitution Is Female. Proc Natl Acad Sci U S A. 1959 Apr; 45(4):554–60. [PubMed: 16590412]
43. Brunkow ME, Jeffery EW, Hjerrild KA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet. 2001 Jan; 27(1):68–73. [PubMed: 11138001]
44. Wildin RS, Ramsdell F, Peake J, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet. 2001 Jan; 27(1): 18–20. [PubMed: 11137992]
45. Bennett CL, Christie J, Ramsdell F, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet. 2001 Jan; 27(1):20–1. [PubMed: 11137993]
46. Anderton SM, Liblau RS. Regulatory T cells in the control of inflammatory demyelinating diseases of the central nervous system. Current opinion in neurology. 2008 Jun; 21(3):248–54. [PubMed: 18451706]
47. Zhang X, Koldzic DN, Izikson L, et al. IL-10 is involved in the suppression of experimental autoimmune encephalomyelitis by CD25+CD4+ regulatory T cells. International immunology. 2004 Feb; 16(2):249–56. [PubMed: 14734610]
48. 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. Journal of immunology. 2002 Nov 1; 169(9):4712–6.
49. Joller N, Lozano E, Burkett PR, et al. Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses. Immunity. 2014 Apr 17; 40(4): 569–81. [PubMed: 24745333]

50. Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. The Journal of experimental medicine. 2004 Apr 5; 199(7):971–9. [PubMed: 15067033]
51. Huan J, Culbertson N, Spencer L, et al. Decreased FOXP3 levels in multiple sclerosis patients. Journal of neuroscience research. 2005 Jul 1; 81(1):45–52. [PubMed: 15952173]
52. Venken K, Hellings N, Thewissen M, et al. Compromised CD4+ CD25(high) regulatory T-cell function in patients with relapsing-remitting multiple sclerosis is correlated with a reduced frequency of FOXP3-positive cells and reduced FOXP3 expression at the single-cell level. Immunology. 2008 Jan; 123(1):79–89. [PubMed: 17897326]
53. Venken K, Hellings N, Broekmans T, Hensen K, Rummens JL, Stinissen P. Natural naive CD4+CD25+CD127low regulatory T cell (Treg) development and function are disturbed in multiple sclerosis patients: recovery of memory Treg homeostasis during disease progression. Journal of immunology. 2008 May 1; 180(9):6411–20.
54. Haas J, Fritzsching B, Trubswetter P, et al. Prevalence of newly generated naive regulatory T cells (Treg) is critical for Treg suppressive function and determines Treg dysfunction in multiple sclerosis. Journal of immunology. 2007 Jul 15; 179(2):1322–30.
55. Borsellino G, Kleinewietfeld M, Di Mitri D, et al. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. Blood. 2007 Aug 15; 110(4):1225–32. [PubMed: 17449799]
56. Mariathasan S, Monack DM. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. Nature reviews Immunology. 2007 Jan; 7(1):31–40.
57. Fletcher JM, Lonergan R, Costelloe L, et al. CD39+Foxp3+ regulatory T Cells suppress pathogenic Th17 cells and are impaired in multiple sclerosis. Journal of immunology. 2009 Dec 1; 183(11): 7602–10.
58. Dalla Libera D, Di Mitri D, Bergami A, et al. T regulatory cells are markers of disease activity in multiple sclerosis patients. PloS one. 2011; 6(6):e21386. [PubMed: 21731726]
59. Muls N, Dang HA, Sindic CJ, van Pesch V. Fingolimod increases CD39-expressing regulatory T cells in multiple sclerosis patients. PloS one. 2014; 9(11):e113025. [PubMed: 25411844]
60. Dominguez-Villar M, Baecher-Allan CM, Hafler DA. Identification of T helper type 1-like, Foxp3+ regulatory T cells in human autoimmune disease. Nature medicine. 2011 Jun; 17(6):673–5.
61. de Andres C, Aristimuno C, de Las Heras V, et al. Interferon beta-1a therapy enhances CD4+ regulatory T-cell function: an ex vivo and in vitro longitudinal study in relapsing-remitting multiple sclerosis. Journal of neuroimmunology. 2007 Jan; 182(1–2):204–11. [PubMed: 17157927]
62. Hong J, Li N, Zhang X, Zheng B, Zhang JZ. Induction of CD4+CD25+ regulatory T cells by copolymer-I through activation of transcription factor Foxp3. Proceedings of the National Academy of Sciences of the United States of America. 2005 May 3; 102(18):6449–54. [PubMed: 15851684]
63. Frisullo G, Nociti V, Iorio R, et al. Regulatory T cells fail to suppress CD4T+-bet+ T cells in relapsing multiple sclerosis patients. Immunology. 2009 Jul; 127(3):418–28. [PubMed: 19016907]
64. Haas J, Korporal M, Balint B, Fritzsching B, Schwarz A, Wildemann B. Glatiramer acetate improves regulatory T-cell function by expansion of naive CD4(+)CD25(+)FOXP3(+)CD31(+) T-cells in patients with multiple sclerosis. Journal of neuroimmunology. 2009 Nov 30; 216(1–2): 113–7. [PubMed: 19646767]
65. Korporal M, Haas J, Balint B, et al. Interferon beta-induced restoration of regulatory T-cell function in multiple sclerosis is prompted by an increase in newly generated naive regulatory T cells. Archives of neurology. 2008 Nov; 65(11):1434–9. [PubMed: 19001161]
66. Kipnis J, Avidan H, Caspi RR, Schwartz M. Dual effect of CD4+CD25+ regulatory T cells in neurodegeneration: a dialogue with microglia. Proceedings of the National Academy of Sciences of the United States of America. 2004 Oct 5; 101(Suppl 2):14663–9. [PubMed: 15331781]
67. Iellem A, Mariani M, Lang R, et al. Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by CD4(+)CD25(+) regulatory T cells. The Journal of experimental medicine. 2001 Sep 17; 194(6):847–53. [PubMed: 11560999]

68. McGeachy MJ, Stephens LA, Anderton SM. Natural recovery and protection from autoimmune encephalomyelitis: contribution of CD4+CD25+ regulatory cells within the central nervous system. Journal of immunology. 2005 Sep 1; 175(5):3025–32.
69. O'Connor RA, Malpass KH, Anderton SM. The inflamed central nervous system drives the activation and rapid proliferation of Foxp3+ regulatory T cells. Journal of immunology. 2007 Jul 15; 179(2):958–66.
70. Korn T, Mitsdoerffer M, Croxford AL, et al. IL-6 controls Th17 immunity in vivo by inhibiting the conversion of conventional T cells into Foxp3+ regulatory T cells. Proceedings of the National Academy of Sciences of the United States of America. 2008 Nov 25; 105(47):18460–5. [PubMed: 19015529]
71. Korn T, Reddy J, Gao W, et al. Myelin-specific regulatory T cells accumulate in the CNS but fail to control autoimmune inflammation. Nature medicine. 2007 Apr; 13(4):423–31.
72. Pot C, Apetoh L, Kuchroo VK. Type 1 regulatory T cells (Tr1) in autoimmunity. Seminars in immunology. 2011 Jun; 23(3):202–8. [PubMed: 21840222]
73. Bacchetta R, Bigler M, Touraine JL, et al. High levels of interleukin 10 production in vivo are associated with tolerance in SCID patients transplanted with HLA mismatched hematopoietic stem cells. The Journal of experimental medicine. 1994 Feb 1; 179(2):493–502. [PubMed: 7905018]
74. Vieira PL, Christensen JR, Minaee S, et al. IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring CD4+CD25+ regulatory T cells. J Immunol. 2004 May 15; 172(10):5986–93. [PubMed: 15128781]
75. Barrat FJ, Cua DJ, Boonstra A, et al. In vitro generation of interleukin 10-producing regulatory CD4(+) T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. The Journal of experimental medicine. 2002 Mar 4; 195(5):603–16. [PubMed: 11877483]
76. Gagliani N, Magnani CF, Huber S, et al. Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. Nature medicine. 2013 Jun; 19(6):739–46.
77. Bacchetta R, Sartirana C, Levings MK, Bordignon C, Narula S, Roncarolo MG. Growth and expansion of human T regulatory type 1 cells are independent from TCR activation but require exogenous cytokines. Eur J Immunol. 2002 Aug; 32(8):2237–45. [PubMed: 12209636]
78. Groux H, O'Garra A, Bigler M, et al. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature. 1997 Oct 16; 389(6652):737–42. [PubMed: 9338786]
79. Magnani CF, Alberigo G, Bacchetta R, et al. Killing of myeloid APCs via HLA class I, CD2 and CD226 defines a novel mechanism of suppression by human Tr1 cells. European journal of immunology. 2011 Jun; 41(6):1652–62. [PubMed: 21469116]
80. Awasthi A, Carrier Y, Peron JP, et al. A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. Nature immunology. 2007 Dec; 8(12):1380–9. [PubMed: 17994022]
81. Fitzgerald DC, Zhang GX, El-Behi M, et al. Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. Nature immunology. 2007 Dec; 8(12):1372–9. [PubMed: 17994023]
82. Stumhofer JS, Silver JS, Laurence A, et al. Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nature immunology. 2007 Dec; 8(12):1363–71. [PubMed: 17994025]
83. Apetoh L, Quintana FJ, Pot C, et al. The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. Nature immunology. 2010 Sep; 11(9):854–61. [PubMed: 20676095]
84. Pot C, Apetoh L, Awasthi A, Kuchroo VK. Molecular pathways in the induction of interleukin-27-driven regulatory type 1 cells. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research. 2010 Jun; 30(6):381–8.
85. Pot C, Jin H, Awasthi A, et al. Cutting edge: IL-27 induces the transcription factor c-Maf, cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. Journal of immunology. 2009 Jul 15; 183(2):797–801.

86. Gandhi R, Kumar D, Burns EJ, et al. Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and Foxp3(+) regulatory T cells. Nature immunology. 2010 Sep; 11(9):846–53. [PubMed: 20676092]

87. Wildbaum G, Netzer N, Karin N. Tr1 cell-dependent active tolerance blunts the pathogenic effects of determinant spreading. The Journal of clinical investigation. 2002 Sep; 110(5):701–10. [PubMed: 12208871]

88. Astier AL, Meiffren G, Freeman S, Hafler DA. Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis. The Journal of clinical investigation. 2006 Dec; 116(12): 3252–7. [PubMed: 17099776]

89. Rovetto MJ, Lamberton WF, Neely JR. Mechanisms of glycolytic inhibition in ischemic rat hearts. Circulation research. 1975 Dec; 37(6):742–51. [PubMed: 157]

90. Martinez-Forero I, Garcia-Munoz R, Martinez-Pasamar S, et al. IL-10 suppressor activity and ex vivo Tr1 cell function are impaired in multiple sclerosis. European journal of immunology. 2008 Feb; 38(2):576–86. [PubMed: 18200504]

91. Ohnmacht C, Pullner A, King SB, et al. Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity. The Journal of experimental medicine. 2009 Mar 16; 206(3):549–59. [PubMed: 19237601]

92. Yamazaki S, Iyoda T, Tarbell K, et al. Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. The Journal of experimental medicine. 2003 Jul 21; 198(2):235–47. [PubMed: 12874257]

93. Darrasse-Jeze G, Deroubaix S, Mouquet H, et al. Feedback control of regulatory T cell homeostasis by dendritic cells in vivo. The Journal of experimental medicine. 2009 Aug 31; 206(9):1853–62. [PubMed: 19667061]

94. Laouar Y, Town T, Jeng D, et al. TGF-beta signaling in dendritic cells is a prerequisite for the control of autoimmune encephalomyelitis. Proceedings of the National Academy of Sciences of the United States of America. 2008 Aug 5; 105(31):10865–70. [PubMed: 18669656]

95. Sela U, Olds P, Park A, Schlesinger SJ, Steinman RM. Dendritic cells induce antigen-specific regulatory T cells that prevent graft versus host disease and persist in mice. The Journal of experimental medicine. 2011 Nov 21; 208(12):2489–96. [PubMed: 22084406]

96. Pflanz S, Timans JC, Cheung J, et al. IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+ T cells. Immunity. 2002 Jun; 16(6):779–90. [PubMed: 12121660]

97. Pflanz S, Hibbert L, Mattson J, et al. WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. Journal of immunology. 2004 Feb 15; 172(4):2225–31.

98. Villarino AV, Huang E, Hunter CA. Understanding the pro- and anti-inflammatory properties of IL-27. Journal of immunology. 2004 Jul 15; 173(2):715–20.

99. Takeda A, Hamano S, Yamanaka A, et al. Cutting edge: role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment. Journal of immunology. 2003 May 15; 170(10):4886–90.

100. Wang S, Miyazaki Y, Shinozaki Y, Yoshida H. Augmentation of antigen-presenting and Th1-promoting functions of dendritic cells by WSX-1(IL-27R) deficiency. Journal of immunology. 2007 Nov 15; 179(10):6421–8.

101. Huber M, Steinwald V, Guralnik A, et al. IL-27 inhibits the development of regulatory T cells via STAT3. International immunology. 2008 Feb; 20(2):223–34. [PubMed: 18156621]

102. Chen Y, Langrish CL, McKenzie B, et al. Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. The Journal of clinical investigation. 2006 May; 116(5):1317–26. [PubMed: 16670771]

103. Yang J, Yang M, Htut TM, et al. Epstein-Barr virus-induced gene 3 negatively regulates IL-17, IL-22 and RORgamma t. European journal of immunology. 2008 May; 38(5):1204–14. [PubMed: 18412165]

104. Batten M, Li J, Yi S, et al. Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. Nature immunology. 2006 Sep; 7(9):929–36. [PubMed: 16906167]

105. Fitzgerald DC, Ciric B, Touil T, et al. Suppressive effect of IL-27 on encephalitogenic Th17 cells and the effector phase of experimental autoimmune encephalomyelitis. Journal of immunology. 2007 Sep 1; 179(5):3268–75.

106. Xu J, Yang Y, Qiu G, et al. c-Maf regulates IL-10 expression during Th17 polarization. Journal of immunology. 2009 May 15; 182(10):6226–36.

107. Mascanfroni ID, Yeste A, Vieira SM, et al. IL-27 acts on DCs to suppress the T cell response and autoimmunity by inducing expression of the immunoregulatory molecule CD39. Nature immunology. 2013 Oct; 14(10):1054–63. [PubMed: 23995234]

108. Pitkanen J, Peterson P. Autoimmune regulator: from loss of function to autoimmunity. Genes and immunity. 2003 Jan; 4(1):12–21. [PubMed: 12595897]

109. Leech MD, Chung CY, Culshaw A, Anderton SM. Peptide-based immunotherapy of experimental autoimmune encephalomyelitis without anaphylaxis. European journal of immunology. 2007 Dec; 37(12):3576–81. [PubMed: 18000952]

110. David A, Crawford F, Garside P, Kappler JW, Marrack P, MacLeod M. Tolerance induction in memory CD4 T cells requires two rounds of antigen-specific activation. Proceedings of the National Academy of Sciences of the United States of America. 2014 May 27; 111(21):7735–40. [PubMed: 24821788]

111. Hilliard B, Ventura ES, Rostami A. Effect of timing of intravenous administration of myelin basic protein on the induction of tolerance in experimental allergic encephalomyelitis. Multiple sclerosis. 1999 Feb; 5(1):2–9. [PubMed: 10096096]

112. Fitzgerald DC, Zhang GX, Yu S, Cullimore ML, Zhao Z, Rostami A. Intravenous tolerance effectively overcomes enhanced pro-inflammatory responses and experimental autoimmune encephalomyelitis severity in the absence of IL-12 receptor signaling. Journal of neuroimmunology. 2012 Jun 15; 247(1–2):32–7. [PubMed: 22522341]

113. Turley DM, Miller SD. Prospects for antigen-specific tolerance based therapies for the treatment of multiple sclerosis. Results and problems in cell differentiation. 2010; 51:217–35. [PubMed: 19130025]

114. Weiner HL, Friedman A, Miller A, et al. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. Annual review of immunology. 1994; 12:809–37.

115. Ratts RB, Arredondo LR, Bittner P, Perrin PJ, Lovett-Racke AE, Racke MK. The role of CTLA-4 in tolerance induction and ttigen administration cell differentiation in experimental autoimmune encephalomyelitis: i. v. antigen administration. International immunology. 1999 Dec; 11(12): 1889–96. [PubMed: 10590254]

116. Jiang Z, Li H, Fitzgerald DC, Zhang GX, Rostami A. MOG(35–55) i.v suppresses experimental autoimmune encephalomyelitis partially through modulation of Th17 and JAK/STAT pathways. European journal of immunology. 2009 Mar; 39(3):789–99. [PubMed: 19224632]

117. Li H, Zhang GX, Chen Y, et al. CD11c+CD11b+ dendritic cells play an important role in intravenous tolerance and the suppression of experimental autoimmune encephalomyelitis. Journal of immunology. 2008 Aug 15; 181(4):2483–93.

118. Smith CE, Eagar TN, Strominger JL, Miller SD. Differential induction of IgE-mediated anaphylaxis after soluble vs. cell-bound tolerogenic peptide therapy of autoimmune encephalomyelitis. Proceedings of the National Academy of Sciences of the United States of America. 2005 Jul 5; 102(27):9595–600. [PubMed: 15983366]

119. Getts DR, McCarthy DP, Miller SD. Exploiting apoptosis for therapeutic tolerance induction. Journal of immunology. 2013 Dec 1; 191(11):5341–6.

120. Getts DR, Turley DM, Smith CE, et al. Tolerance induced by apoptotic antigen-coupled leukocytes is induced by PD-L1+ and IL-10-producing splenic macrophages and maintained by T regulatory cells. Journal of immunology. 2011 Sep 1; 187(5):2405–17.

121. Zhang Y, Kim HJ, Yamamoto S, Kang X, Ma X. Regulation of interleukin-10 gene expression in macrophages engulfing apoptotic cells. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research. 2010 Mar; 30(3):113–22.

122. Lutterotti A, Yousef S, Sputtek A, et al. Antigen-specific tolerance by autologous myelin peptide-coupled cells: a phase 1 trial in multiple sclerosis. Science translational medicine. 2013 Jun 5.5(188):188ra75.

123. Hunter Z, McCarthy DP, Yap WT, et al. A biodegradable nanoparticle platform for the induction of antigen-specific immune tolerance for treatment of autoimmune disease. ACS nano. 2014 Mar 25; 8(3):2148–60. [PubMed: 24559284]

124. Ilarregui JM, Croci DO, Bianco GA, et al. Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10. Nature immunology. 2009 Sep; 10(9):981–91. [PubMed: 19668220]

125. Toscano MA, Bianco GA, Ilarregui JM, et al. Differential glycosylation of TH1, TH2 and TH-17 effector cells selectively regulates susceptibility to cell death. Nature immunology. 2007 Aug; 8(8):825–34. [PubMed: 17589510]

126. Kopcow HD, Rosetti F, Leung Y, Allan DS, Kutok JL, Strominger JL. T cell apoptosis at the maternal-fetal interface in early human pregnancy, involvement of galectin-1. Proceedings of the National Academy of Sciences of the United States of America. 2008 Nov 25; 105(47):18472–7. [PubMed: 19011096]

127. Rabinovich GA, Toscano MA. Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. Nature reviews Immunology. 2009 May; 9(5):338–52.

128. Wang J, Lu ZH, Gabius HJ, Rohowsky-Kochan C, Ledeen RW, Wu G. Cross-linking of GM1 ganglioside by galectin-1 mediates regulatory T cell activity involving TRPC5 channel activation: possible role in suppressing experimental autoimmune encephalomyelitis. Journal of immunology. 2009 Apr 1; 182(7):4036–45.

129. Garin MI, Chu CC, Golshayan D, Cernuda-Morollon E, Wait R, Lechler RI. Galectin-1: a key effector of regulation mediated by CD4+CD25+ T cells. Blood. 2007 Mar 1; 109(5):2058–65. [PubMed: 17110462]

130. Cedeno-Laurent F, Opperman M, Barthel SR, Kuchroo VK, Dimitroff CJ. Galectin-1 triggers an immunoregulatory signature in Th cells functionally defined by IL-10 expression. Journal of immunology. 2012 Apr 1; 188(7):3127–37.

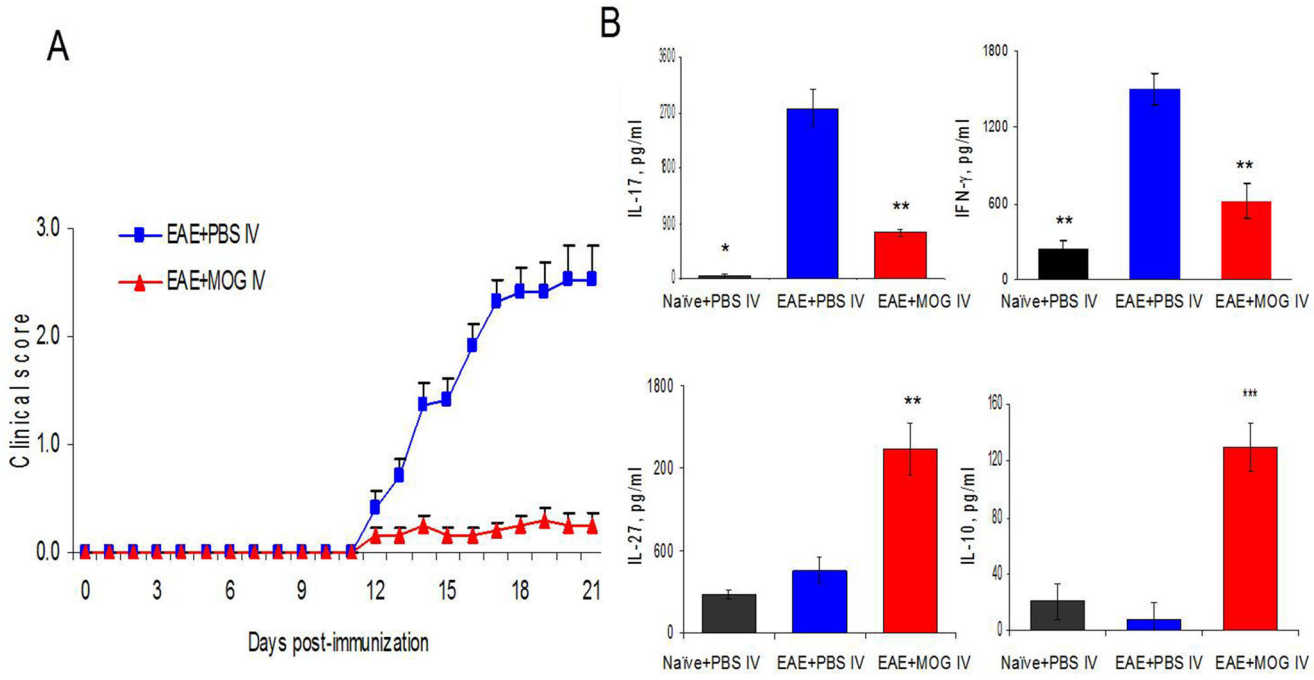

Figure 1.

Myelin oligodendrocyte glycoprotein ($MOG_{35–55}$) i.v. inhibited the development of experimental autoimmune encephalomyelitis (EAE). EAE was induced in C57BL/6 mice by immunization with $MOG_{35–55}$/CFA, and pertussis toxin was given on days 0 and 2. At days 0, 3 and 6 p.i., mice were i.v. injected with 200 μg $MOG_{35–55}$ to induce tolerance with the same volume of phosphate-buffered saline (PBS) to serve as control. (a) Daily clinical scores of each mouse group (10 mice each group; $P < 0.01$). (b) Splenocytes in duplicate from mice in (a) were isolated at day 21 p.i., and cultured with 10 μg/mL $MOG_{35–55}$ for 72 h. Production of interleukin (IL)-17, interferon (IFN)-γ, IL-27p28 and IL-10 in supernatants was analyzed by enzyme-linked immunosorbent assay. Data were pooled from two independent experiments and presented as mean value ± SEM ($n = 10$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.