

Multiple sclerosis immunology

The healthy immune system vs the MS immune system

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ABSTRACT

Multiple sclerosis (MS) is a debilitating autoimmune disease characterized by both inflammation and axonal degeneration. The resulting demyelination and subsequent degeneration of axons account for the disability of patients with MS. Early investigations indicated that disease progression was driven by CD4⁺ effector T cells. However, clinical therapies specifically targeting these cells have, for the most part, not been effective. Therefore, new areas of research in experimental autoimmune encephalomyelitis (the experimental model of MS) and human MS have identified previously unknown contributions to disease pathogenesis, including interleukin-17-producing T helper 17 cells, B cells, CD8⁺ T cells, and both CD4⁺ and CD8⁺ T-regulatory cells. Research into the respective mechanisms of action of these cells has identified novel therapeutic targets to combat this devastating disease. This article reviews the autoimmune response in patients with MS compared with individuals without MS and summarizes the fundamental differences in the immunologic response between people with and without MS. Investigations into these autoimmune differences and the disruption of the homeostatic balance of the immune system will help guide future research into MS therapeutics, with particular attention to the long-term management of this disease. **NEUROLOGY 2010;74(Suppl 1):S2-S8**

GLOSSARY

AIS = adaptive immune system; **APC** = antigen-presenting cells; **BAFF** = B-cell-activating factor; **CSF** = cerebral spinal fluid; **DC** = dendritic cell; **EAE** = experimental autoimmune encephalomyelitis; **IFN** = interferon; **IL** = interleukin; **MHC** = major histocompatibility complex; **MS** = multiple sclerosis; **MyD** = myeloid differentiation factor; **NK** = natural killer; **T regs** = T-regulatory cells; **TCR** = T-cell receptor; **TGF** = transforming growth factor; **Th** = T helper; **TLR** = toll-like receptor.

INTRODUCTION The immune response in patients with multiple sclerosis (MS) differs from the regulation and activation of the reaction in nonaffected individuals. However, identification of the disrupted critical pathways that lead to the development of MS has shown that the progression of this disease is multifaceted. Experimental autoimmune encephalomyelitis (EAE), the animal model for human MS, has long been considered the best experimental model to study MS. Much literature on the EAE model has been generated on the role of T cells, in particular CD4⁺ T cells polarized to a T helper (Th)1 phenotype, in driving the autoimmune process. Based on this model, clinical research in MS has primarily focused on aberrant CD4⁺ T-cell actions in MS. However, the ineffective clinical results

specifically targeting CD4⁺ T-cell biology, such as anti-interleukin (IL)12p40, have begun to shift the collective thinking away from CD4⁺ T cells and toward other cell types that contribute either to the pathogenesis of MS, such as Th17 cells or B cells, or to the suppression of these cells by T-regulatory cells (T regs). Understanding the role of these cells in normal and diseased CNS tissue has led to the development of new agents that target specific pathways to treat MS. This review focuses on some of the key players of the immune system and identifies the targets of several MS therapies.

COMPONENTS OF THE ADAPTIVE IMMUNE SYSTEM The innate immune system protects the body from potentially harmful substances by pattern

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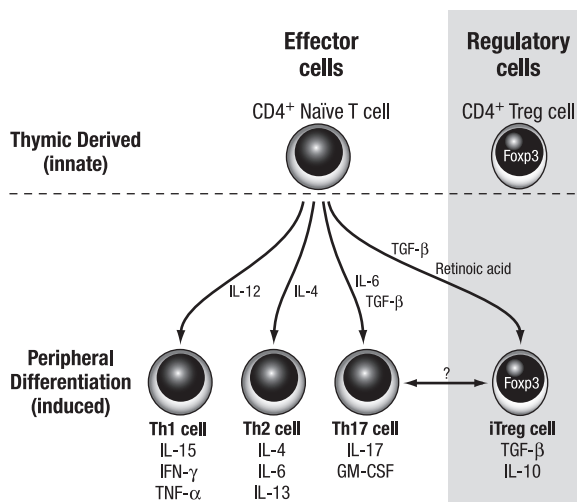
recognition and responding to microbial antigens via constitutive expression of various toll-like receptors (TLRs). The adaptive immune system (AIS) is characterized by the production of signature antibodies that serve as an “immunologic memory” and thus “remember” each pathogen. The AIS comprised mostly lymphocytic B cells, which produce the antibodies that attach to specific antigens, and T cells. T cells attack antigens directly by the release of ILs, which control the immune response.

Antigen-presenting cells (APCs), which express special immunostimulatory receptors known as co-stimulatory molecules on their cell surfaces, are specifically designed to present antigens and to prime naïve T cells. Various types of APCs exist, including dendritic cells (DCs), monocytes, macrophages, and B cells. DCs are the primary APCs and can be further classified into 2 cell types: myeloid or plasmacytoid. Myeloid DCs are tissue cells found throughout the body as interstitial cells or as Langerhan cells found in the skin. Plasmacytoid DCs are found in the bone marrow and secrete interferon (IFN)- β on activation.¹ In addition to DCs, monocytes circulate throughout the body, monitoring for inflammatory

signals. When inflammation is present, monocytes can convert into CD11c⁺ DCs or macrophages. Macrophages, including tissue-fixed microglial cells, phagocytose cellular debris or pathogens. Epithelial cells that line the gut, or enterocytes, also have the capacity to present antigens. Once these professional APC cells capture a pathogen, they display foreign antigens complexed with major histocompatibility complexes (MHCs) on their surface to enable recognition of the antigen by T cells.

The interaction between the APC and T cell is an essential component to mount the adaptive immune response. Several types of T cells including CD4⁺ and CD8⁺ phenotype can be activated by APCs, although T cells themselves are not capable of antigen presentation. Th1 or Th2 cells are CD4⁺ effector cells that are polarized in response to exposure to specific ILs. Once polarized to either Th1 or Th2, these effector T cells continue to express and secrete specific ILs. For example, an uncommitted CD4⁺ T cell on exposure to IL-12 will be polarized to a Th1 cell and begin to secrete IFN- γ (figure 1). In contrast, the same uncommitted CD4⁺ T cell exposed to IL-4 becomes polarized to a Th2 cell and begins to secrete IL-4 and IL-13. Depending on the disease process, both of these populations exhibit effector functions. Th1 cells are involved in MS pathogenesis and Crohn disease, whereas Th2 cell involvement has been implicated in asthma and ulcerative colitis. In MS, Th2 cells exhibit regulatory function as Th2 polarized cells. Natural or adaptive CD25⁺CD4⁺ T regs are a special subpopulation of T cells that suppress activation of the immune system and maintain immune homeostasis. These cells also express Foxp3, a critical transcription factor that controls development and function of T regs.² A recently identified Th subset, IL-17-expressing Th17 cells, becomes differentiated in the presence of transforming growth factor (TGF)- β , IL-1, IL-6, IL-21, and IL-23.³ The conditions for this differentiation differ somewhat between humans and other species. In addition to the T cells, there are a number of other effector cell populations that appear to be involved in the pathogenesis of MS, including CD56⁺ natural killer (NK) cells, invariant NK T cells that express both NK and T-cell markers, and stem cells. Adult stem cells are classified into 3 distinct categories: hemapoietic stem cells, mesenchymal stem/stromal cells, and endothelial progenitor cells.⁴

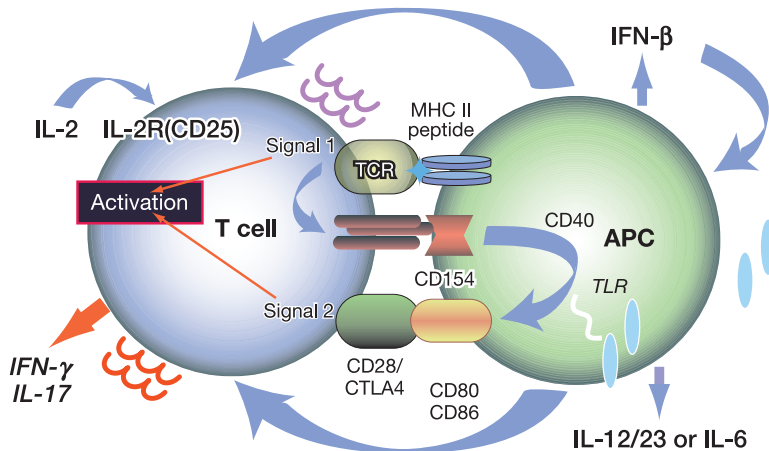
Figure 1 Differentiation of CD4⁺ T cells



On exposure to interleukin (IL)-12, naïve CD4⁺ T cells can polarize to a Th1 phenotype associated with the production of specific cytokines; the most prominent of which are interferon-gamma (IFN- γ) or tumor necrosis factor-alpha (TNF α). If a naïve CD4⁺ T cell is exposed to IL-4, it will polarize to a T helper (Th)2 phenotype associated with production of cytokines such as IL-13. In multiple sclerosis (MS), it has long been postulated based on the experimental autoimmune encephalomyelitis (EAE) model that Th1 CD4⁺ T cells are inflammatory and Th2 CD4⁺ T cells are regulatory. In addition, there is increasing evidence that Th17 cells and T-regulatory cells are critical to immune homeostasis. If a naïve CD4⁺ T cell is exposed to both IL-16 and transforming growth factor (TGF)- β , the naïve cell will polarize to a Th17 cell associated with the secretion of IL-17 and granulocyte-macrophage colony-stimulating factor (GM-CSF) that are inflammatory in several experimental autoimmune conditions, including EAE, the animal model for human MS. However, if the naïve CD4⁺ T cell is exposed to TGF- β in combination with retinoic acid, it will polarize to a T-regulatory cell associated with expression of the nuclear protein Foxp3. These cells have the capacity to secrete both IL-10 and TGF- β that are regulatory in EAE. Figure concept courtesy of Jennifer Joscelyn and Lloyd H. Kasper.

OVERVIEW OF ANTIGEN PRESENTATION The innate immune response is triggered by the recognition by immune cells of nonself-antigens such as microbial products. The distinction between the body's cells and unwanted foreign invaders becomes ob-

Figure 2 Interactions between antigen-presenting cells and T cells in the activation of the immune response



Pattern recognition molecules, called toll-like receptors (TLR), are found on the surface of antigen-presenting cells (APCs) and identify very specific microbial ligands when activated. On activation, the APC interacts with a T-cell receptor (TCR) on the surface of the T cell and, if the APC is a CD4 cell, identifies a class II molecule and activates signal 1. Then, CD154 becomes upregulated on the surface of the APC and interacts with constitutively expressed CD40 on the surface of the APC. The combination of these interactions drives the upregulation of CD80 and CD86 on the surface of the APC. These molecules then interact with CD28 and CTLA4 on the surface of the T cell to yield signal 2, which activates the T cell (which releases IFN- γ and interleukin [IL]-17) and leads to the secretion of cytokines (IL-12/23 or IL-6) by the APC.

scured in autoimmune diseases. On the surface of APCs are pattern recognition molecules, of which, TLRs are most studied. The function of these pattern recognition receptors is to identify very specific microbial products. On ligation, the APC is activated by the NF- κ B pathway that leads to the production of type 1 IFNs, including IFN- β .⁵ Once activated, APCs interact with CD4⁺ T cells through T-cell receptors (TCRs) that recognize MHC class II molecules on the APCs (figure 2), which leads to the induction of the adaptive immune response. This first interaction between the TCR and peptide-loaded MHC class II (or class I in the case of CD8⁺ T cells) is referred to as signal 1. Recognition of the TCR/MHC alone is insufficient to drive T-cell activation. If the cascade ended after this initial signal, the T cell would become anergic, and the APC would fail to express or secrete ILs that will further activate the T cell. Under normal physiologic conditions, this first signal upregulates the CD40 ligand, CD154, on the surface of the T cell, which binds to its receptor. CD40 is constitutively expressed on APCs such as B cells and DCs. The combination of this interaction further drives the upregulation of both CD80 and CD86 on the surface of APCs. CD80 and CD86 identify 2 additional receptors, CD28 and CTLA4, on the surface of the T cells to provide a second signal to APCs. CD28 is associated with activation of the T cell, whereas CTLA4 is more

regulatory. Other costimulatory molecules either amplify or regulate this interaction between APCs and T cells. After this second signal, the T cells become activated, and APCs begin to secrete important cytokines, including IL-12 and IL-23, which bind to specific receptors on T cells and drive them to secrete different cytokines, such as IFN- γ or IL-17, depending on the cytokine milieu. T cells also begin to secrete IL-2, which then activates its own receptor, CD25 or the IL-2 receptor. During the activation of T cells, there is an upregulation on their surface of α 4 β 1, an important ligand involved in trafficking and passage of the lymphocytes through the blood-brain barrier, which is the target of natalizumab.^{6,7}

Another protein upregulated on the surface of T cells, sphingosine-1-phosphate-1 receptors, are critical for the egress of T cells from lymph nodes and are controlled by a gradient of sphingosine-1-phosphate in the serum.⁸ If the APC is a CD11b-expressing, plasmacytoid DC, it begins to secrete type 1 IFNs, which drive the T cell toward a further activation state.¹ Many of the novel therapeutics currently in development for treatment of MS and other autoimmune conditions are targeting a variety of molecules involved in this costimulatory or signal transduction cascade.

ACTIVATED DCs AND TLRs IN CNS DEMYELINATING DISEASE

TLRs have been shown to be involved in the pattern recognition of very specific microbial products, and all but one are dependent on myeloid differentiation factor (MyD) 88.^{9,10} The downstream effect of engaging TLRs by MyD88 is the activation of NF- κ B and the production of IFN- β , which is a potent proinflammatory molecule in this model.¹⁰ However, recent studies in a murine EAE model have suggested that physiologic doses of IFN- β may also exhibit antiinflammatory effects.¹¹ Nevertheless, once TLRs on DCs are engaged, the DCs can become activated.

When activated through TLRs, DCs become semimature and have the capacity to induce T regs to produce inhibitory cytokines such as IL-10 or TGF- β .¹² As the DCs continue to mature, they develop the capacity to polarize CD4⁺ T cells to differentiate into either Th1 or Th2 phenotypes.¹³ DCs in the cerebral spinal fluid (CSF) circulation also appear to survey inflamed CNS tissue and migrate to periventricular demyelinating lesions and cervical lymph nodes.¹⁴ TLR-9 and MyD88 are essential mediators for the induction of EAE, which suggests that endogenous danger signals are involved in the modulation of disease pathogenesis.¹⁵ In addition, TLR-4 ligation has been shown to prevent EAE development by promoting Th1 differentiation, which may inhibit

Th17 differentiation.¹⁶ This *in vivo* research suggests that DCs in MS are probably in an activated state and may be the primary source of disease pathogenesis. Recent findings indicate that glatiramer acetate can affect DC activation and subsequently skew the immune system to promote a Th2 bias and development of T regs, which ameliorated the disease in mice.¹⁷ Similarly, it has been shown that IFN- β can also skew T-cell polarization toward a Th2 bias along with increased expression of IL-10 and concomitant downregulation of the strongly polarized Th1 cytokine, IFN- γ .

T-CELL FUNCTION IN DEMYELINATING DISEASE

Evidence has suggested that Th1 polarized CD4⁺ T cells are the effector cells in MS that contribute to immune pathogenesis as they do in the EAE model.¹⁸ Adoptively transferring Lyt1⁺ T cells (which are functionally equivalent to human CD4⁺ cells) from an EAE mouse to a naïve mouse induces paralysis, confirming that these cells are critical to EAE development.¹⁹ If Th1 polarized CD4⁺ T cells do drive the disease, it would be possible to shift the balance between CD4⁺ effector cells and regulatory cells to induce T regs to control aberrant autoimmune responses by suppressing Th1 cell effector functions. In contrast, there is reasonable clinical evidence that does not support a pivotal role for CD4⁺ T cells in MS disease pathogenesis. Patients treated with a monoclonal anti-CD4⁺ antibody in a phase II trial realized no benefit.²⁰ No therapeutic benefit was found in a phase II anti-IL-12p40 neutralizing antibody clinical trial.²¹ Uncertain benefits have been reported from a CD4⁺ T-cell vaccine trial.²² The first CTLA4-immunoglobulin clinical trial indicated only a modest clinical benefit.²³ Furthermore, treatment with 2 altered peptide ligands for myelin basic peptide actually exacerbated MS symptoms for some patients, and as a result, both trials had to be terminated early.^{24,25} Taken together, these clinical trial data suggest that in contrast to EAE, inhibiting T-cell function may not be a good therapeutic target to control the pathogenesis of MS.

Another cell type that has been implicated in MS disease progression is the IL-17-producing Th17 cell. As mentioned above, if naïve CD4⁺ cells are exposed to IL-6, TGF- β , and IL-21, they become Th17 cells that are maintained by the production of IL-23.^{3,26} IL-27, an IL-12/IL-23 family member, is a negative regulator of Th17 differentiation^{3,11} and moreover can prevent inflammatory demyelination in the EAE model. Th17 cells are responsible for producing IL-17 and perhaps IL-22, which are involved in the migration and trafficking of neutrophils to a site.^{26,27} Although IL-22 is coexpressed with IL-17, the spe-

cific role in disease pathogenesis remains uncertain.²⁷ That Th17 cells appear to be absolutely essential to drive the disease process in EAE is a paradigm shift away from a classic Th1/Th2 polarized disease.²⁸ In acute and chronic MS plaques, IL-17 receptors are identified immunohistochemically in patients with MS but are not found in controls.²⁹ MS endothelial cells express high levels of IL-17 receptors and are more permeable in response to IL-17, suggesting that these cells are actively involved in the promotion of CNS inflammation.²⁸ Increased levels of IL-17 expression can be identified in the peripheral blood mononuclear cells of patients during disease exacerbation³⁰ and are decreased in response to high dose IFN- β and glatiramer acetate, as determined by the surrogate marker CCR6 and CD161.³¹

The fourth cell type to be differentiated from a naïve CD4⁺ cell is the T reg, which matures when it is exposed to TGF- β and the vitamin A precursor retinoic acid. T regs can be maintained with retinoic acid, and they produce TGF- β and IL-10.³² T regs are not only primarily focused on the regulation of IL-17 T cells but can also regulate the function of effector Th1/Th2 cells. Although there is no difference in the number of T regs between MS and control patients, patients with MS do demonstrate reduced T reg function and are not able to suppress T reg responses and controls.³³ Both high-dose IFN- β and glatiramer acetate have the capacity to enhance T reg numbers and function in EAE and MS.³⁴⁻³⁶ Alemtuzumab, a therapy currently under evaluation, appears to be able to influence the production of T regs and may shift the unbalanced regulatory/effector relationship found in MS back into homeostasis.^{37,38} Various ways have been identified to influence the production of T regs, including the presence of cytokines (TGF- β , IL-10, and blockade of IL-9), the blockade of costimulatory molecules such as CD154, CD52, CD20, and S1P, and treatment with immune modifiers. Cortisol, retinoic acid, and T-cell vaccines modulate the numbers of T regs,²² and glatiramer acetate and IFN- β have been shown to induce populations of T regs.^{17,35}

CD8⁺ T cells found in MS lesions might also have a regulatory function in MS disease progression. These cells demonstrate both immune suppressive and pathogenic characteristics and are prevalent in MS lesions.³⁹⁻⁴¹ CD8⁺ cells can mediate suppression on CD4⁺ lymphoproliferation through the secretion of perforin, which is cytotoxic and leads to CD4⁺ cell inactivation.⁴⁰ Indirectly, CD8⁺ cells interact with APCs that mediate CD4⁺ cell unresponsiveness. CD8⁺ cells can also directly influence neuropathology through the recognition of class I peptide epitopes on the surfaces of axons. These cells can also

kill glial cells, leaving the axons exposed. CD8⁺ cells can transect axons, induce oligodendrocyte death, and promote vascular permeability, all of which are observed in MS lesions. Even though they have the capacity to damage, CD8⁺ cells also have the ability to suppress injury, implying that mechanisms involving CD8⁺ function are important to understanding their role in MS.

B-CELL FUNCTION IN MS There is an evolving paradigm shift in our understanding of the function of B cells in neurologic disease. It was previously thought that B cells simply become plasma cells that make antibodies. Antibody accumulation and secretion as oligoclonal bands or increased IgG synthesis within the CSF compartment has been long recognized as a clinical characteristic to assist in disease diagnosis. The specific function or target of these secreted antibodies has not been revealed. However, in addition to maturing into antibody producing plasma cells, B cells have the robust capacity to present antigen and interact with T cells.⁴² Moreover, B cells are abundant producers of both proinflammatory (IFN- γ) and regulatory (IL-10) cytokines. In addition, B cells have the capacity to form ectopic lymphoid structures or germinal centers, which have been seen in patients with rheumatoid arthritis and MS.⁴²⁻⁴⁵ B cells begin as stem cells and progress through several antigen-independent maturation stages until they become mature-but-naïve B cells.⁴² Once they are exposed to an antigen, they differentiate into activated B cells, memory B cells, late plasmoblasts, and plasma cells. B cells express CD19 until the late plasmoblast stage. CD20, the target of rituximab, is present throughout most of the B-cell maturation process but is not present on plasma cells.

There is increasing evidence that B cells and their products are involved in MS pathogenesis and that treatment targeting these cells may provide long-lasting benefits. A recent report provides evidence that B-cell follicles are found in the brains of patients with secondary-progressive MS and that these follicles express CD20⁺.⁴⁵ CD20⁺ B cells in the plasmoblast stage make autoimmune antibodies, and their migration to the CNS is dependent on CXCL13 and $\alpha 4\beta 1$.⁴² Treatment with anti-CD20 (rituximab or the more recently formulated, fully humanized, ocrelizumab) activates complement factors to attack the lipid raft environment on CD20⁺ B cells and induces apoptosis. In turn, this can allow for the recruitment of macrophages and NK cells. In both a phase I trial and a 48-week phase II trial, rituximab significantly reduced inflammatory brain lesions and clinical relapses.^{46,47} The proposed mechanism of in-

hibition of B-cell antigen presentation is particularly attractive, given that CD20⁺ B cells are depleted for up to 12 months after rituximab infusion.⁴² Consideration must also be given for a possible role by plasma cells in immune regulation and homeostasis, such that depletion of the B-cell compartment (other than memory B cells) by anti-CD20 may lead to disease stabilization.

Another approach is to target B-cell-activating factor (BAFF), which is one of the cytokines responsible for B-cell maturation. BAFF levels are increased in the spinal fluid of patients with MS during relapse.⁴⁸ In addition, BAFF levels are also increased in the brains of people with MS.⁴⁹ BAFF drives B-cell activation and may be crucial for the development of immune responses.⁵⁰ Various modulators of BAFF are currently undergoing clinical trials.⁵⁰

It is of interest that the common denominator for many of the newer, seemingly more robust therapeutic agents coming down the development pipeline is B-cell depletion or immune alteration to a more regulatory cytokine profile. This includes anti-CD52 (alemtuzumab), anti-CD20 (rituximab and ocrelizumab), FTY 720 (fingolimod), ONO-4641, and others. The effects of the currently available disease-modifying therapies, including IFN- β , glatiramer acetate, and natalizumab, on B-cell function are now coming to be better appreciated. B cells as a therapeutic target are even more of interest because, as aforementioned, no T-cell-specific therapeutic has shown much clinical efficacy to date in either phase II or phase III trials.

CONCLUSION The realization that MS is not strictly dependent on Th1/Th2 mechanisms has opened the door to promising new targets for successful MS treatment. The heterogeneity of this disease and the interplay of multiple cell types with pleiotropic effects explain, in part, why research delving into this disease has not produced an effective long-term treatment. We are now coming to better appreciate that although EAE is a reasonable model to study immune interactions at the basic level, it does not necessarily reflect a realistic portrait of disease pathogenesis in humans. However, the clues that have been provided by the EAE model have lead us to our current success in treating this complex immunologic disease. As we begin to extend our findings beyond the now classic and perhaps historic approach of Th1/Th2 polarization and investigate the role of other immune compartments, including Th17 cells, T regs, CD8⁺ T cells, DCs, and B cells in this disease, we will be able to find new targets for therapeutic intervention. For example, recent research suggests that the proinflammatory cytokine,

osteopontin, plays a role in the pathogenesis of both EAE and MS and may be an important target.^{51–54} Of importance will be the identification of the source for these circulating effector cells and the mechanisms by which these cells traffic from the periphery into the CNS.

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