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# THE ROLE OF CD4 T CELLS IN THE PATHOGENESIS OF MULTIPLE SCLEROSIS

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T lymphocytes play a central role in the pathogenesis of multiple sclerosis (MS) (Zhang *et al.*, 1992). Both CD4+ and CD8+ T cells have been demonstrated in MS lesions, with CD4+ T cells predominating in acute lesions and CD8+ T cells being observed more frequently in chronic lesions (Raine, 1994). Additionally, T cells are found in all four of the described histopathologic subtypes of MS (Lucchinetti *et al.*, 2000). Activated myelin-reactive CD4+ T cells are present in the blood and cerebrospinal fluid (CSF) of MS patients; in contrast, only nonactivated myelin-reactive T cells are present in the blood of controls (Zhang *et al.*, 1994). The success of several T-cell-targeted therapies in MS reinforces the importance of the role

of the T cell in MS pathogenesis. Here, we outline basic concepts in CD4+ T-cell immunology and summarize the current understanding of the role of CD4+ T cells in the pathogenesis of MS.

## I. Overview of CD4+ T-Cell Immunology

### A. THE T-CELL SUBSET OF INFLAMMATORY CELLS

T cells as well as B cells are critical components of the adaptive immune system. Both T cells and B cells are equipped with specific antigen recognition receptors, the former with the T-cell receptor (TCR), while B cells secrete immunoglobulin (Ig) molecules. T cells originate and differentiate in the thymus. Every T cell that leaves the thymus is conferred with a unique specificity for recognizing antigens through its TCR. The TCR consists of two glycosylated polypeptide chains, the alpha ( $\alpha$ ) and beta ( $\beta$ ) chains, which are linked by disulfide bonds. Each chain consists of variable (V), joining (J), and constant (C) regions closely resembling Ig chains. T cells that recognize self-antigens with high affinity are either deleted or rendered tolerant within the thymus, through a process called central tolerance.

### B. SUBSETS OF T CELLS

T cells may be divided into two groups on the basis of their expression of either the CD4+ or the CD8+ surface molecules. Functionally, CD4+ T cells are involved in delayed-type hypersensitivity (DTH) responses and also provide help for B-cell differentiation, and hence are termed T helper (Th) cells. In contrast, CD8+ T cells are involved in class I-restricted lysis of antigen-specific targets, and hence are termed cytotoxic T cells. The CD4 molecule binds to a nonpolymorphic site on the major histocompatibility complex (MHC) class II  $\beta$  chain that is expressed by antigen-presenting cells (APCs). In contrast CD8 binds to the  $\alpha$ -3 domain of the MHC class I molecule expressed by most cell types. The MHC molecule serves to present antigen to the T cell via the TCR.

### C. ACTIVATION OF T CELLS

Signaling through surface molecules by second messengers deliver signals for cell division to the nucleus. The CD3 molecule is part of the TCR complex, although the TCR interacts with the MHC-peptide complex on APCs, signals for the subsequent enactment of T-cell activation and proliferation are delivered

by the CD3 antigen. The cytoplasmic tail of the CD3 proteins contains one copy of a sequence motif important for signaling functions, called the immunoreceptor tyrosine-based activation motif (ITAM). Phosphorylation of the ITAM initiates intracellular signaling events. The interaction of MHC-peptide complex with T cells, while necessary, is insufficient for T-cell activation. Additional classes of molecules are involved in T-cell antigen recognition, activation, intracellular signaling, adhesion, and trafficking of T cells to their target organs.

Two signals are required for T lymphocyte activation. According to this “two-signal” model (Bretscher and Cohn, 1970), “signal 1” consists of the interaction of the TCR with antigen, presented by the MHC on the surface of APCs. “Signal 2” consists of the engagement of costimulatory receptors on the T cell by ligands present on the surface of APCs (Alegre *et al.*, 2001; Bretscher, 1999). After contact with specific antigen-MHC complex and adequate costimulatory signals, T cells begin to proliferate, differentiate, and deliver a series of signals, enabling effector functions to other cells such as B cells and NK cells. T cells can thereby orchestrate the immune response.

Costimulatory molecules may deliver either a stimulatory (positive) or an inhibitory (negative) signal for T-cell activation (Brunet *et al.*, 1987). Examples of molecules delivering a positive costimulatory signal for T-cell activation include the B7-CD28 and CD40-CD154 pathways. Examples of molecular pathways delivering a negative signal for T-cell activation include B7-CTLA4 and PD1-PD ligand. The delicate balance between positive and negative regulatory signals can determine the outcome of a specific immune response. Importantly, in the absence of adequate costimulatory signals, T cells can die or become anergic *in vitro*, and thus, fail to initiate an effective immune response *in vivo*. Therefore, manipulation of costimulatory signals represents an important mechanism to inhibit immune-activation.

#### D. MEMORY T CELLS

On exposure to an antigen, antigen-specific T cells proliferate and differentiate into effector T cells (Sprent and Surh, 2002). The vast majority of effector T cells undergo apoptosis as the immune response progresses, and the few lymphocytes that survive become long-lived memory T cells (Dutton *et al.*, 1998). Memory T cells are specific to the antigen encountered during the primary immune response and react rapidly and vigorously on reencounter with the same antigen. Functionally, in terms of activation requirements, memory T cells can be activated by lower concentrations of anti-CD3 (Byrne *et al.*, 1988), require less costimulation by anti-CD28 (Kuiper *et al.*, 1994), and readily secrete more effector cytokines (Bird *et al.*, 1998; Ehlers and Smith, 1991; Lee *et al.*, 1990) than naive T-cell counterparts, indicating a state of hyperresponsiveness.

## E. MIGRATION OF T CELLS

Molecules primarily involved in cell migration into tissues include chemokines, integrins, selectins, and matrix metalloproteinases (MMPs). Chemokines constitute a large family of chemoattractant peptides that regulate the vast spectrum of leukocyte migration events through interactions with chemokine receptors. The integrin family includes vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and leukocyte function antigen 3 (LFA-3), CD45, and CD2. The integrin family also mediates T-cell adhesion, facilitates interaction with the APCs, as well mediates adhesion to nonhematopoietic cells such as endothelial cells and guides cell traffic. L-Selectins facilitate the rolling of leukocytes along the surface of endothelial cells and function as a homing receptor to target peripheral lymphoid organs. The MMPs are a family of proteinases, secreted by inflammatory cells, which digest specific components of the extracellular matrix, thereby facilitating lymphocyte entry through basement membranes, including the blood-brain barrier (BBB).

## F. T-CELL CYTOKINE PRODUCTION

The Th cells play a critical role in the orchestration of the immune response, in part, through the production of cytokines that provide secondary signals to other cells in the immune cascade. Two major types of Th cell responses have been described. Th1 cells produce IL-2, TNF- $\alpha$ , and interferon (IFN)- $\gamma$ , while Th2 cells produce IL-4, IL-5, IL-10, and IL-13. A Th3 cell that primarily secretes TGF- $\beta$  has been described in the context of oral tolerance to myelin antigens (Fukaura *et al.*, 1996; Hafler *et al.*, 1997) and in other immune-mediated settings (Minguela *et al.*, 1999). These subsets of T cells interregulate each other's development, with Th1 cytokines suppressing Th2 differentiation and vice versa (Fig. 1).

A subset of T cells that predominantly produces IL-17 has been described (Yao *et al.*, 1995). These cells are believed to represent a distinct subset from IFN- $\gamma$ -producing Th1 cells, evidenced by the dependence of Th<sub>IL-17</sub> cells on IL-16 and TGF- $\beta$  for differentiation (Bettelli *et al.*, 2006; Mangan *et al.*, 2006; Veldhoen *et al.*, 2006) and IL-23 for expansion (Aggarwal *et al.*, 2003; Langrish *et al.*, 2005), as opposed to Th1 cells which are dependent on IL-12 and IL-2, respectively, for differentiation and expansion. Both Th1 and Th2 cytokines have been shown to suppress the development of Th17 cells (Harrington *et al.*, 2005; Park *et al.*, 2005). An intriguing relationship between the generation of pathogenic Th17 and regulatory CD4+CD25+Foxp3+ cells has recently been demonstrated. TGF- $\beta$  is critical for the differentiation and generation of CD4+CD25+Foxp3+ regulatory T cells; however, if additionally exposed to IL-6 during culture, these cells differentiate into Th17 cells (Bettelli *et al.*, 2006).

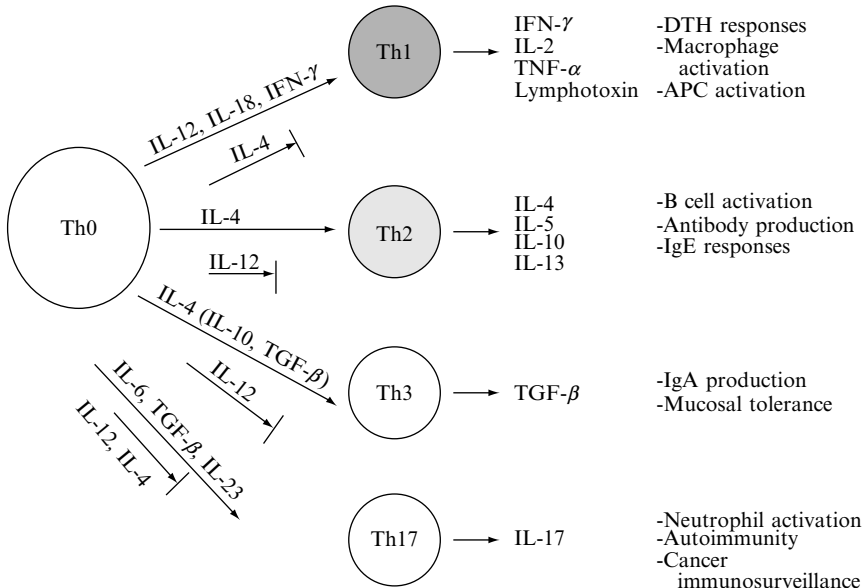


FIG. 1. Factors that influence T helper cell differentiation.

Traditionally, Th cell subsets have been distinguished by their patterns of cytokine production, however identification of distinguishing surface molecule markers has been a major advance in the field. T-cell-, Ig-, and mucin-domain-containing molecules (Tim) represent an important family of molecules which encode cell-surface receptors involved in the regulation of Th1- and Th2-cell-mediated immunity. Tim-3 is specifically expressed on Th1 cells and negatively regulates Th1 responses through interaction with the Tim-3 ligand, galactin-9, also expressed on CD4+ T cells (Monney *et al.*, 2002; Sabatos *et al.*, 2003; Zhu *et al.*, 2005). Tim-2 is expressed on Th2 cells (Chakravarti *et al.*, 2005) and appears to negatively regulate Th2 cell proliferation, although this has not been fully established. Tim-1 is expressed on Th2 cells > Th1 cells and interacts with Tim-4 on APCs to induce T-cell proliferation (Meyers *et al.*, 2005).

### G. T-CELL-SIGNALING PATHWAYS

Intracellular signaling mechanisms provide the link between the binding of the cytokine with its receptor and the effect of the cytokine on cellular function. The Janus kinase and signal transducer and activator of transcription (Jak/STAT) family of transducer/transcription-activating factors plays a critical role in the

signaling of many cytokine receptors. Cytokine binding to the specific receptor activates the Jak molecule associated with the receptor, causing phosphorylation of tyrosine residues, and binding of the Jak molecules to its receptor. This facilitates binding of STAT proteins to the phosphorylated receptor, which subsequently dissociates from the receptor, dimerizes, and activates transcription of genes containing specific *cis*-regulatory STAT-binding sequences. Different cytokine receptors are associated with different Jak/STAT proteins. The IL-12 receptor is associated with Jak-2 and STAT3 and STAT4 (Jacobson *et al.*, 1995). The IL-4 receptor is associated with Jak-1–3 and STAT6 (Kaplan *et al.*, 1996; Takeda *et al.*, 1996) (Fig. 1). Mice deficient in STAT6 display a reduction in Th2 cytokine production, decreased IL-4-induced B-cell proliferation and reduced IgE (Kaplan *et al.*, 1996; Takeda *et al.*, 1996). In contrast, STAT4 plays a pivotal role in Th1 immune responses. STAT4 is activated after IL-12 interacts with the IL-12 receptor, inducing transcription of IFN- $\gamma$  (Jacobson *et al.*, 1995). Mice deficient in STAT4 lack IL-12-induced IFN- $\gamma$  production and Th1 differentiation (Kaplan *et al.*, 1996; Thierfelder *et al.*, 1996), and display a predominantly Th2 phenotype (Kaplan *et al.*, 1996).

T-bet is a Th1-specific T box transcription factor that directly controls the expression of the hallmark Th1 cytokine, IFN- $\gamma$ , and IL-12R $\beta$ 2 expression, thus facilitating Th1 cell differentiation (Szabo *et al.*, 2000). The transcription factor c-maf enhances IL-4 production and represents an important step in the induction of Th2 cells (Ho *et al.*, 1996). GATA-3 was found to be an STAT6-independent inducer of Th2 differentiation, and is located upstream of c-maf, thus representing a master switch in Th2 development and commitment (Ouyang *et al.*, 2000).

Th17 differentiation is independent of STAT4 and STAT6 signaling (Park *et al.*, 2005); however, it has been demonstrated that STAT3 signaling is activated by both IL-6 and IL-23, and binds to IL-17 gene promoters (Chen *et al.*, 2006). SOCS-3 is a major regulator of IL-23-mediated STAT3 phosphorylation and subsequent Th17 generation (Chen *et al.*, 2006).

## H. REGULATORY T CELLS

Several populations of regulatory or suppressor T cells have been described in humans. These include CD4+CD25+Foxp3 regulatory T cells (Baecher-Allan *et al.*, 2001; Dieckmann *et al.*, 2001; Levings *et al.*, 2001; Stephens *et al.*, 2001; Yagi *et al.*, 2004), CD8+CD28<sup>-</sup> T cells (Koide and Engleman, 1990), IL-10-producing Th2 cells (Bacchetta *et al.*, 1994), and TGF- $\beta$ -producing Th3 cells (Kitani *et al.*, 2000; Roncarolo and Levings, 2000). Regulatory T cells suppress T-cell proliferation through a variety of mechanisms, including the production of immunosuppressive cytokines, or through T–T-cell interactions. Several studies have demonstrated that these cells play an important role in the control of the immune

response in multiple sclerosis (MS) and that the function of regulatory T cells may be enhanced by immunomodulatory therapies (Crucian *et al.*, 1995; Fukaura *et al.*, 1996; Hafler *et al.*, 1997; Karaszewski *et al.*, 1991; Vigiuetta *et al.*, 2004).

## II. T-Cell Immunologic Studies in MS

CD4+ T cells are believed to play a central role in the pathogenesis of MS. In this section, we summarize the prevailing theory of the pathogenesis of MS, and evidence for the role of CD4+ T cells from both human MS and animal models of disease.

### A. ANIMAL MODELS OF MS

Much of the current understanding of the potential mechanistic and role of CD4+ T cells in MS comes from the animal models simulating features of MS. Experimental autoimmune encephalomyelitis (EAE) is an inflammatory central nervous system (CNS) demyelinating disease, and may be induced in several animal types via immunization with myelin proteins or peptides. Disease is primarily mediated by myelin-reactive Th1 cells, which precipitate an inflammatory, demyelinating response within the CNS (Chitnis *et al.*, 2001b). Transfer of myelin basic protein (MBP)-specific T-cell clones restricted to class II (Ia) antigens of the MHC into naive recipient animals causes a similar inflammatory demyelinating disease (Zamvil *et al.*, 1985). EAE reproduces many of the clinical and immunologic aspects of MS, and has been widely used to study the mechanisms of CD4+ T-cell priming and response to myelin components (Bettelli *et al.*, 1998; Chitnis *et al.*, 2001a) as well as to test potential therapies for MS (Aharoni *et al.*, 1999; Yednock *et al.*, 1992).

Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) model is a virally mediated model of CNS inflammatory demyelination, with some resemblance to MS and is induced by direct CNS infection of the neurotropic TMEV picornavirus, initially resulting in an immune-mediated reaction primarily involving TMEV-specific CD4 and CD8 T cells (Clatch *et al.*, 1986; Rodriguez *et al.*, 1996). However, during the chronic stages of disease, T-cell reactivity to host myelin peptides has been observed, indicating epitope spreading has occurred, causing secondary T-cell responses to myelin breakdown products, and resulting in a disseminated autoimmune response (Miller *et al.*, 1997).

A summary of T-cell immunology related to animal models of MS is beyond the scope of this chapter, and can be found elsewhere in this volume or in alternate sources (Chitnis and Houry, 2003a,b); however, selected topics in MS that are illuminated by animal studies are discussed.



## B. MOLECULAR MIMICRY AND THE INITIATION OF AN IMMUNE RESPONSE IN MS

The prevailing theory of the etiology of MS is that of “molecular mimicry” whereby CD4+ T cells activated by a foreign antigen cross-react with myelin antigens. Activated myelin-reactive CD4+ T cells are present in the blood and cerebrospinal fluid (CSF) of MS patients; in contrast, only nonactivated myelin-reactive T cells are present in the blood of controls (Zhang *et al.*, 1994). Sequences in MBP has been shown to resemble several viral sequences, and in some cases, cross-reactive T-cell responses have been demonstrated. Although no pathogen has definitely been proven to be the cause of MS, it is conceivable that certain pathogens serve as molecular mimics to CNS components or play a role in the activation of myelin-specific CD4+ T cells. Examples of cross-reactive T cells with MBP antigens include *human herpesvirus 6* (HHV-6) (Tejada-Simon *et al.*, 2003), staphylococcal enterotoxin antigens (Zhang *et al.*, 1995), coronavirus (Talbot *et al.*, 1996), influenza virus hemagglutinin (Markovic-Plese *et al.*, 2005), and Epstein-Barr virus (EBV) (Lang *et al.*, 2002). Proteolipid protein (PLP) shares common sequences with *Haemophilus influenzae* (Olson *et al.*, 2001), while *Semliki Forest virus* (SFV) peptides mimic epitopes of myelin oligodendrocyte glycoprotein (MOG) (Mokhtarian *et al.*, 1999).

These activated T cells are then thought to migrate to the CNS, where they undergo reactivation in response to nascent myelin antigens. The reactivation of T cells heralds an inflammatory response within the CNS, resulting in more tissue damage and release of secondary antigens. Subsequent T-cell reactivity to secondary antigens is termed “epitope spreading.” Evidence of epitope spreading has been demonstrated in animal models of MS (McMahon *et al.*, 2005; Vanderlugt *et al.*, 1998), and may play an important role in the pathogenesis of the human disease.

Although this is the most widely accepted paradigm of MS pathogenesis, and is supported by evidence from studies discussed in this chapter, there still remain many unanswered questions, which include the identity of the initiating foreign cross-reactive antigen(s), the identity of the initiating self-antigen directed T-cell response, and the nature of epitope spreading within the CNS. Moreover, the paucity of CD4+ T cells in certain pathological subtypes of MS questions whether alternate mechanisms may predominate in subsets of this heterogeneous disease.

## C. CD4+ T CELLS IN THE PERIPHERAL IMMUNE SYSTEM OF MS PATIENTS

Because of the focus on myelin proteins and, in particular, MBP as a potential autoantigen in MS (Allegretta *et al.*, 1990; Chou *et al.*, 1992; Zhang *et al.*, 1994), considerable interest has developed in the role of T-cell responses to MBP. Moreover, MS disease-associated MHC class II allele, DRB1\*1501 has been shown to be effective in presenting MBP peptide to T-cell clones isolated from MS patients (Wucherpfennig *et al.*, 1995, 1997). Activated myelin-reactive CD4+ T cells

are present in the blood and CSF of MS patients; in contrast, only nonactivated myelin-reactive T cells are present in the blood of controls (Zhang *et al.*, 1994). Furthermore, MBP-reactive T cells isolated from the CSF of MS patients display increased expression of the IL-2 receptor (Zhang *et al.*, 1994), consistent with a previously activated or memory phenotype. In MS patients, but not in healthy controls, these cells can be activated in the absence of CD28-B7 costimulation, thus implying that they have been previously activated *in vivo* (Markovic-Plese *et al.*, 2001; Scholz *et al.*, 1998). MBP-reactive T cells from MS patients were found to be less responsive to CTLA-4 blockade compared to those from healthy controls (Oliveira *et al.*, 2003), signifying that in MS patients these T cells are not subject to the normal regulatory mechanisms. Although the contribution of MBP-reactive T cells to the pathogenesis of MS is currently unknown, their differential phenotype and costimulatory requirements indicate a memory and potentially dysregulated cell population.

T-cell reactivity to other myelin proteins and peptides in MS has been explored. Studies examining PLP T-cell responses demonstrated T-cell proliferation to certain epitopes (Pelfrey *et al.*, 1994), with some differential reactivity when compared to controls (Markovic-Plese *et al.*, 1995; Zhang *et al.*, 1994). T-cell responses to recombinant MOG appeared to be similar in MS patients and healthy controls (Diaz-Villoslada *et al.*, 1999), although other studies demonstrated increased reactivity (Kerlero de Rosbo *et al.*, 1993) or altered T-cell properties (Van der Aa *et al.*, 2003a) in MS patients. Other studies have examined T-cell responses to myelin oligodendrocyte basic protein (MOBP) (Holz *et al.*, 2000) or 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) (Muraro *et al.*, 2002), with some reactivity demonstrated in T-cell lines isolated from select MS patients. T-cell reactivity to other CNS antigens has not been fully explored due to the technical difficulties with performing and interpreting the results of such assays. Many studies currently employ strategies to expand T cells using nonspecific methods or mixtures of myelin peptides.

Studies in patients with postinfectious encephalomyelitis or acute disseminated encephalomyelitis (ADEM) have consistently found robust T-cell reactivity to myelin peptides in both the blood and CSF (Hafler *et al.*, 1987; Hemachudha *et al.*, 1988; Pohl-Koppe *et al.*, 1998), and suggest an intriguing relationship in the pathophysiology of ADEM and MS.

#### D. TCR REPERTOIRES IN MS

Studies examining TCR repertoire in MS patients have demonstrated a bias for use of  $\beta$  chain variable region ( $V\beta$ ) 5.2 and 5.3 (Kotzin *et al.*, 1991; Lozeron *et al.*, 1998; Oksenberg *et al.*, 1993), and this has led to the exploration of TCR  $V\beta$  5.2/5.3-targeted therapies. TCR vaccines employing TCR  $V\beta$  5.2 peptides are

thought to exert their effects by enhancing the function of regulatory T-cell populations recognizing TCR determinants (Vandenbark, 2005; Vandenbark *et al.*, 1996), and are currently undergoing pilot studies in MS. Phase II trials using antibodies specifically targeting the V $\beta$  5.2/5.3 sequence of the TCR have shown some success and no significant adverse effects (Killestein *et al.*, 2002; Olsson *et al.*, 2002). However, other groups (Gran *et al.*, 1998; Lozeron *et al.*, 1998; Musette *et al.*, 1996) have found predominant usage of other V $\beta$  chains, indicative of potential limitations with TCR V $\beta$  5.2-targeted therapies. Studies in twins demonstrated similar selection of TCR V $\alpha$  chains in concordant twins in response to MBP, when compared to discordant twins or controls, suggesting a genetic basis for the evolution of self-antigen T-cell responses (Utz *et al.*, 1993). MS patients treated with autologous hematopoietic stem cell transplantation demonstrated an increase in naive compared to memory CD4+ T cells, with increased TCR diversity indicative of broader clonal phenotypes 2 years following therapy (Muraro *et al.*, 2005). A separate study ascertained similar findings and found that MBP-reactive T cells demonstrated broader epitope recognition following reconstitution (Sun *et al.*, 2004).

#### E. T CELLS IN MS LESIONS

Pathologically, MS lesions are characterized by perivascular infiltrates of CD4+ and CD8+ T cells and macrophages (Prineas and Wright, 1978; Traugott *et al.*, 1983b). CD4+ and CD8+ T cells and macrophages are also found toward the periphery of the lesion and in the normal appearing white matter (Traugott *et al.*, 1983a). CD4+ T cells were shown to predominate in acute lesions, while CD8+ T cells were observed more frequently in chronic lesions (Raine, 1994). Similar densities of CD3+ T cells have been demonstrated in all four of the histopathologic subtypes of MS, although type III and IV lesions are additionally characterized by prominent oligodendrocyte degeneration (Lucchinetti *et al.*, 1996). Attempts to isolate T-cell clones from the brains of MS patients failed to show either MBP or PLP reactivity (Hafler *et al.*, 1987). TCR analysis from MS lesions demonstrated a broad TCR V $\alpha$  and V $\beta$  repertoires in active lesions, while fewer TCR V genes were detected in chronic plaques and control samples (Wucherpfennig *et al.*, 1992). Other studies demonstrated restricted TCR specificities, with rearranged V $\beta$  5.2 genes found in the brains of all patients who were HLA DRB1\*1501, DQA1\*0102, DQB1\*0602, and DPB1\*0401 positive, suggesting that MHC class II genotype may play a role in VDJ rearrangements in MS lesions (Oksenberg *et al.*, 1993). T cells in parenchymal MS lesions lacked CCR7, indicating a differentiation of central-memory T cells into effector memory cells presumably on restimulation by antigen within the CNS (Kivisakk *et al.*, 2004).

In summary, multiple studies have demonstrated the presence of CD4+ T cells in MS lesions, arguing for a central role in MS pathogenesis. The lack of

consensus regarding T-cell specificities suggest a heterogeneity in T-cell responses at the time of analysis, which may be a result of epitope spreading in chronic disease.

#### F. T-CELL ACTIVATION AND COSTIMULATION

Costimulatory pathways deliver a positive or negative signal for T-cell activation, and thus represent an important control step in the immune response. The CD28/CTLA-4-B7-1/2 family of costimulatory molecules represents an important step in the activation of CD4+ T cells. Lesions in the CNS of patients with MS were found to be exclusively associated with the expression of B7-1 in perivascular lymphocytes, while B7-2 was expressed on macrophages in both MS and in other neurological diseases (Windhagen *et al.*, 1995). Peripheral blood mononuclear cells (PBMCs), isolated from MS patients, showed increased expression of B7-1 on both CD4+ and CD8+ cell patients with rapidly progressive disease, compared to those with stable disease, or normal controls (Mena and Rohowsky-Kochan, 1999). In a separate study, B7-1 expression localized to B cells was found to be increased during MS relapses, and treatment with IFN- $\beta$  1b reduced the number of B7-1-expressing B cells but increased the number of B7-2 monocytes (Genc *et al.*, 1997). In total, these observations suggest that the B7-CD28-CTLA4 pathway is activated in MS, and that B7-1, in particular, may play an important role in regulating disease activity.

Genetic polymorphisms of costimulatory molecules may contribute to disease susceptibility. Three CTLA-4 gene polymorphisms were found in MS patients, but not in healthy controls (Ligers *et al.*, 1999), however no association was found with disease course or severity (Masterman *et al.*, 2002). In an Olmstead County study, two polymorphisms were associated with the presence of MS (Kantarci *et al.*, 2003). The Canadian Collaborative Study found no association of CTLA-4 polymorphisms with the disease course of MS (Dyment *et al.*, 2002). The exon 1 A/G polymorphism was associated with the presence of oligoclonal bands in the CSF (Fukazawa *et al.*, 1999). Thus, dysregulation of CTLA-4 signaling may contribute to susceptibility to MS. A phase I safety study of CTLA4Ig (Repligen-RG2077) as well as a multicenter study of CTLA4Ig (BMS-188667) for MS are ongoing.

Interaction of CD40 on APCs with CD154 on T cells induces APC production of IL-12, a major factor in Th1 cell differentiation (Kelsall *et al.*, 1996). Expression of both CD40 and CD154 were increased in lesions from postmortem MS brains compared with controls, with CD40 found predominantly on macrophages and microglia, while CD154 colocalized with the CD4 T-cell marker (Gerritse *et al.*, 1996). Expression of CD154 was found to be higher in peripheral blood monocytes isolated from SPMS compared with RRMS or

healthy controls (Filion *et al.*, 2003; Jensen *et al.*, 2001), and was reduced by IFN- $\beta$  treatment (Teleshova *et al.*, 2000). PBMCs from SPMS patients produced more IL-12 and IFN- $\gamma$  when restimulated *in vitro*, compared with healthy controls (Karni *et al.*, 2002). In summary, these studies indicate that the CD40-CD154 pathway is important for the regulation of Th1 cytokine production in MS. Clinical trials with an anti-CD154 antibody (Biogen) in autoimmune disease such as ITP and lupus were terminated because of the occurrence of thromboembolic events. Another formulation of the antibody (IDEC pharmaceuticals) is under investigation. A phase I clinical trial in MS patients was recently performed with good safety data, and therapeutic effects are currently under investigation.

Programmed death-1 (PD-1) is expressed on T cells and is a negative regulator of T-cell activation. PD-1 polymorphism was shown to be a genetic modifier of the progression of MS, and this may relate to PD-1-mediated inhibition of T-cell activation (Kroner *et al.*, 2005).

The Tim family of molecules are important cell-surface markers as well as costimulatory regulators of Th1/Th2 responses. In MS patients, CSF T-cell clones demonstrated reduced levels of Tim-3 and T-bet and secreted higher amounts of IFN- $\gamma$  than did those from control subjects, indicating that Tim-3 may represent an important regulator of Th1 responses in MS (Koguchi *et al.*, 2006).

In summary, there is significant evidence that costimulatory molecules represent an important step in the control of T-cell activation in MS and are viable therapeutic targets.

## G. T-CELL CYTOKINE PRODUCTION IN MS

In the context of MS, Th1 cytokines are thought to mediate disease, while Th2 cytokines are believed to play a protective role. However, as our understanding of the disease evolves, it is clear that this paradigm is not absolute. Moreover, evidence of a distinct lineage of Th17-producing T cells has led to reevaluation of the role of Th1 cytokines in MS.

Th1 cytokines are predominantly found in the brains of MS patients, while a paucity of Th2 cytokines, in particular TGF- $\beta$ , and IL-10 was observed (Cannella and Raine, 1995b; Hofman *et al.*, 1986; Woodroffe and Cuzner, 1993). Studies using semiquantitative RT-PCR and immunocytochemistry found increased expression of B7-1 and IL12p40 in acute MS plaques, compared with samples isolated from inflammatory infarcts (Windhagen *et al.*, 1995). IL-6, IFN- $\gamma$ , and TNF- $\alpha$  were expressed by cells located in the perivascular cuffs, suggesting that in acute MS lesions, inflammatory cells are the most important source of these cytokines (Woodroffe and Cuzner, 1993). Expression of TNF- $\alpha$  was localized to macrophages, microglia, and astrocytes (Cannella and Raine, 1995b;

Hofman *et al.*, 1989; Selmaj *et al.*, 1991) in chronic-active lesions. A separate study found that IL-2 was expressed predominantly in association with perivascular inflammatory cells examining acute MS lesions (Hofman *et al.*, 1986). Contrary to the Th1/Th2 paradigm of MS, high levels of IL-4 were expressed in both acute and chronic-active MS lesions with no obvious correlation to the resolution of the lesion (Cannella and Raine, 1995b). Studies using RNA microarrays in MS brains at autopsy found increased transcripts of genes encoding for IL-6, IL-17, and IFN- $\gamma$  (Lock *et al.*, 2002), indicating a potential role for both Th1 and Th17 cells in proinflammatory responses in MS.

Several studies have demonstrated enhanced production of the hallmark Th1 cytokine, IFN- $\gamma$ , from PBMCs restimulated *ex vivo* in MS patients compared to controls (Balashov *et al.*, 1997; Comabella *et al.*, 1998). Clinical attacks correlated with increased IFN- $\gamma$  production *in vitro* (Beck *et al.*, 1988). In a similar study, IFN- $\beta$  was found to blunt increased production of IFN- $\gamma$  during relapse (Becher *et al.*, 1999). TCR-mediated IFN- $\gamma$  and IL-10 secretions are increased in relapsing-remitting (RR) and secondary progressive (SP) patients, but not in primary progressive disease, suggesting a dysregulation of this signaling pathway in certain MS subtypes (Balashov *et al.*, 2000). Interestingly, SPMS patients also exhibit seasonal variations of IFN- $\gamma$  production with increased expression in autumn and winter months compared with spring and summer months, which was not observed in normal controls (Balashov *et al.*, 1998). A progressive course of MS was found to be significantly more frequent in carriers of the IFN- $\gamma$  receptor-2 allele Arg64 (Schrijver *et al.*, 2004). Administration of IFN- $\gamma$  to MS patients precipitated clinical attacks, confirming the role of IFN- $\gamma$  as a proinflammatory cytokine in MS (Panitch *et al.*, 1987a,b).

Studies of the prototypic Th2 cytokine IL-4 in MS are limited, IL-4 was expressed in high levels in both acute- and chronic-active MS lesions (Cannella and Raine, 1995a). High frequencies of T-cell clones reactive to MBP- and PLP-expressing IL-4 were found in MS patients compared with untreated patients (Chou *et al.*, 1992). Increased expression of IL-4 secretion by CD3-stimulated PBMCs was demonstrated in SPMS patients treated with cyclophosphamide/methylprednisolone compared with untreated patients (Smith *et al.*, 1997). Thus, the role of IL-4 in the pathogenesis of MS is unclear, however may be associated with responses to therapy.

Studies examining Th17 cell activity in MS found that dendritic cells from MS patients secrete elevated amounts of IL-23 and express increased levels of IL-23p19 mRNA, and are associated with increased T-cell production of IL-17 (Vaknin-Dembinsky *et al.*, 2006). A Japanese study examining cytokine expression found that CSF levels of IL-17, IL-8, and IL-5 were significantly higher in opticospinal-MS patients than in conventional RRMS patients, and may be associated with pronounced neutrophilic infiltrates typically found in the opticospinal disease variant (Ishizu *et al.*, 2005).

## H. REGULATORY T CELLS IN MS

Alterations in the function of several populations of regulatory T cells have been demonstrated in MS. Induction of regulatory antigen-specific Th3 cells through oral tolerance with myelin proteins has been described (Fukaura *et al.*, 1996; Hafler *et al.*, 1997). Deficiency in the CD8+CD28– subset of suppressor cells has been demonstrated in MS patients (Crucian *et al.*, 1995). Moreover, increases in  $\beta$ -adrenergic receptor density on CD8+CD28– cells were found in MS compared with controls (Karaszewski *et al.*, 1991). Much attention has focused on the CD4+CD25+ subset of regulatory T cells. Induction of CD4+CD25+ T cells is controlled by the transcription regulator FOXP3 which also serves as a cell-specific marker (Hori *et al.*, 2003). Defects in the effector function of CD4+CD25+ regulatory T cells have been demonstrated in MS patients (Viglietta *et al.*, 2004). Thus, defects in regulatory T-cell populations may facilitate the development and/or progression of autoimmunity.

## I. T-CELL MIGRATION IN MS

T-cell migration into the CNS in MS is believed to follow the sequence of capture, rolling, activation, adhesion strengthening, and finally transmigration through the BBB. The specifics of these events may vary depending on the region of the CNS, as well as the activation state of T cells and microvasculature.

Two processes appear to be important in T-cell migration into the CNS: (1) migration of T cells from the blood into the CSF with interactions with dendritic elements on the luminal surface of the choroid plexus resulting in immunosurveillance and (2) T-cell migration through inflamed endothelial BBB and interactions with perivascular APCs within the Virchow-Robbins space (Engelhardt and Ransohoff, 2005). Evidence exists for both of these processes in MS, however the relative contribution of each is unclear, and may depend on the stage and subtype of disease.

Adhesion molecule ICAM-1 is expressed on the inflamed BBB in MS, while LFA-1 is expressed on infiltrating T cells, suggesting an important role for this pathway in T-cell migration in MS (Bo *et al.*, 1996). Inhibition of interactions between integrin molecule  $\alpha 4\beta 1$  present on the surface of T cells with VCAM-1 present on endothelial cells of the BBB was shown to suppress the development of EAE (Yednock *et al.*, 1992). This led to the development of an  $\alpha 4\beta 1$ -integrin antibody, natalizumab, which has been effective in reducing MS relapses (Miller *et al.*, 2003). However, clinical trials with a natalizumab led to several cases of progressive multifocal leukoencephalopathy, resulting in the reevaluation of the role of such drugs in MS therapeutics (Kleinschmidt-Demasters and Tyler, 2005; Langer-Gould *et al.*, 2005; Van Assche *et al.*, 2005).

CXCR3 is postulated to be the major chemokine involved in the trafficking of T cells from the blood to the CSF for immunosurveillance in both normals and MS patients (Kivisakk *et al.*, 2002). Studies of chemokine expression on peripheral blood T cells in MS patients found a positive correlation between CXCR3 expression on blood T cells (Eikelenboom *et al.*, 2002) and CSF T cells (Sindern *et al.*, 2002) on MRI measures of disease activity in MS patients.

T-cell migration is an important step in the pathogenesis of MS, as confirmed by the success of  $\alpha 4\beta 1$ -integrin antibody therapy (natalizumab). However, effective blockade of CNS immunosurveillance has produced profound adverse effects, as evidenced by the development of PML in three treated patients. The risk of infection and emergence of tumors due to blockade of immunosurveillance must be balanced with therapeutic effect when utilizing such powerful therapeutics.

## J. T-CELL INTERACTION WITH AXONS AND NEURONS

Much attention has focused on the presence of axonal damage in MS plaques as a substrate for chronic progressive disease (Trapp *et al.*, 1998, 1999). Although mediators of axonal damage include cytokines, complement, antibody, and nitric oxide, T cells may play a significant role both in neurodegeneration and in neuroprotection. The presence of CD8+ T cells, but not CD4+ T cells, in the MS lesion correlates with axonal damage (Bitsch *et al.*, 2000). In the TMEV model of MS, demyelination but no axonal damage was found in the CNS of MHC class I-deficient mice after infection with Theiler's virus (Rivera-Quinones *et al.*, 1998), suggesting that axonal damage is class I mediated. These mice also displayed a deficiency of CD8-positive cells in their CNS lesions. Similarly, *in vitro*, T cell-mediated neurotoxicity was dependent on IFN- $\gamma$ -induced expression of MHC class I (Medana *et al.*, 2001). Collectively, these studies suggest that class I expression in the CNS, particularly on neurons, may enhance a cytotoxic CD8+ T-cell response.

*In vitro* coculture of human fetal neurons with OKT3-activated CD4+ or CD8+ T cells has been found to produce apoptosis of neurons (Giuliani *et al.*, 2003). This process required physical contact of the cells, as demonstrated by transwell experiments, and was not dependent on MHC I. Protection could be conferred by blocking CD40 on both T cells and neurons, and FasL on neurons. Attention has focused on the regulatory role that neurons may play on T cells. In a Lewis rat model of EAE, motoneurons were demonstrated to engulf T lymphocytes through a process consistent with emperipolesis (Smith *et al.*, 2000). Neuronal production of TGF- $\beta$  has been shown to play a significant role in the induction of CD4+CD25+Foxp3+ regulatory T cells in a murine EAE model (Liu *et al.*, 2006). These studies have important implications for understanding the T cell-neuronal interactions in MS.



Although MBP-reactive T cells have been implicated in the pathogenesis of MS (Zhang *et al.*, 1992, 1994), an intriguing study has shown that passive transfer of MBP-reactive T cells resulted in protection of the retinal ganglion cell body after optic nerve crush injury *in vivo* (Moalem *et al.*, 1999). Interestingly, transfer of T cells specific for other antigens was not protective. Furthermore, expression of mRNA for several nerve growth factors, including brain-derived neurotrophic factor (BDNF), was upregulated after antigen-activation of these T cells, while the injured nerve expressed mRNA for nerve growth factor receptors (Moalem *et al.*, 2000). Two major types of Th cell responses have been described, based on cytokine secretion. Th1 cells produce the cytokines IFN- $\gamma$  and TNF- $\alpha$ , while Th2 cells produce IL-4, IL-5, and IL-10. EAE has been associated with a Th1 response, while Th2 cytokines are generally protective. In support of the above findings, a separate study demonstrated that the presence of myelin-reactive Th2 cells conferred neuroprotection in organotypic hippocampal slice cultures (Wolf *et al.*, 2002). These findings have several implications for MS. First, myelin-reactive T cells may be neuroprotective under certain conditions including trauma, suggesting that the MS brain may be more susceptible to inflammation-induced damage. Second, the local production of neurotrophic factors by inflammatory cells may be neuroprotective. However, in contrast to these findings, other studies have shown that MBP-TCR transgenic mice sustained more CNS damage and inflammation following traumatic spinal cord injury, compared with wild-type controls (Jones *et al.*, 2002). T-cell infiltrates were localized to areas of demyelination and axonal loss, suggesting that autoreactive T cells can trigger autoimmune demyelination in the setting of trauma under certain circumstances. Therefore, the therapeutic potential of neuroprotective T lymphocytes should be entertained only with caution.

Glatiramer acetate (GA) is an established treatment for RRMS. Its mechanism of action relies, in part, on the migration of Th2 cells into the CNS (Aharoni *et al.*, 2000, 2002), where they presumably downregulate local inflammatory responses. Interestingly, these T cells have been shown to produce BDNF in the EAE model (Aharoni *et al.*, 2003), and GA-reactive T cells harvested from MS patients treated with GA produce BDNF on restimulation *in vitro* (Ziemssen *et al.*, 2002). However, the role of BDNF in neuroprotection in MS or EAE has not been established. Thus, the role of this and other neurotrophic factors requires further exploration.

### III. T-Cell-Targeted Therapies in MS

The success of several T cell-targeted therapies in MS reinforces the importance of the role of the T cell in MS pathogenesis. Of the six approved therapies for MS, the effects of GA and natalizumab can be directly related to modulation

of T-cell function. The  $\beta$ -interferons (IFN- $\beta$ 1a and IFN- $\beta$ 1b) modulate some T-cell functions including T-cell migration and Th1 cytokine production (Yong, 2002), while mitoxantrone is a cytotoxic agent that nonspecifically abrogates T- and B-cell proliferation.

An altered peptide ligand (APL) may be defined as “any peptide that serves as a receptor ligand in which substitutions of a single or multiple amino acids lead to changes in the functional outcome of receptor signaling” (Bielekova and Martin, 2001). APLs have most commonly been used as TCR ligands to alter T-cell responses to presumed immunogenic or target antigens presumably resulting in immune suppression or immune deviation as well as induction of a regulatory T-cell population reactive to the APL itself, which then serves to downregulate the inflammatory disease process through bystander suppression.

Glatiramer acetate (GA; Copolymer-1; Copaxone<sup>®</sup>) is an FDA approved therapy for the treatment of RRMS. GA is an APL that was originally developed to mimic MBP. It is composed of a random sequence of the amino acids glutamic acid, lysine, alanine, and tyrosine present in a specific molar ratio (0.14:0.34:0.43:0.09). Copaxone is administered by daily subcutaneous injection, and in a phase III clinical trial was found to reduce relapse frequency by 29%, as well as decrease the incidence of new gadolinium enhancing lesions on MRI (Ge *et al.*, 2000; Johnson *et al.*, 1995, 2001). Despite its crude resemblance to MBP, evidence from several studies showing GA stimulates several nonmyelin antigen T-cell lines suggests that GA acts as a “universal” or degenerate T-cell antigen (Duda *et al.*, 2000). GA has been shown to inhibit responses to MBP-specific T-cell lines *in vitro* (Racke *et al.*, 1992), and *in vivo* treatment with GA induces a hyporesponsiveness to this antigen (Schmied *et al.*, 2003). Interestingly, GA-reactive T-cell lines isolated from both treated patients as well as untreated controls were found to cross-react with a variety of peptides, suggesting degenerate antigenicity. In addition, Th2 cytokine deviation was noted in GA-reactive T-cell lines (Duda *et al.*, 2000). In the EAE model, Th2-producing GA-reactive T cells were shown to accumulate in the CNS and attenuate disease (Aharoni *et al.*, 2000). Thus, the principal mechanism of action of Copaxone may be the induction of Th2 responses, which exert bystander suppression of inflammation within the CNS.

APLs targeting MBP have been widely studied in MS because of the interest in this potential autoantigen. An APL to MBP<sub>87–99</sub> peptide was shown to be effective in ameliorating disease in the EAE model (Karin *et al.*, 1994). An initial phase I clinical trial tested four doses of an APL to MBP<sub>83–99</sub> administered subcutaneously for 4 weeks demonstrated no safety concerns (Bielekova and Martin, 2001). Two phase II trials using MBP<sub>83–99</sub> APLs were initiated: A small NIH-based trial tested the highest dose of APL CGP77116 (50 mg) administered weekly for 9 months. Three of eight patients developed atypical MS exacerbations, characterized by a high gadolinium-enhancing lesion load, tumefactive-type lesion, or a flaccid paralysis with inflammatory involvement of the peripheral

nervous system (Bielekova *et al.*, 2000). 2/3 of exacerbations, correlated with enhanced reactivity to MBP (Bielekova *et al.*, 2000). A second larger multicenter study testing three doses of APL NBI-5788 (5, 20, or 50 mg) versus placebo in 144 total patients was terminated because of the occurrence of APL-induced systemic hypersensitivity reactions in 9% of enrolled patients (Kappos *et al.*, 2000). In both studies, enhanced *ex vivo* T-cell responses to APL were observed following treatment. In patients who developed hypersensitivity reactions, enhanced Th2 responses to the APL could be demonstrated (Kappos *et al.*, 2000). Further phase II studies investigating the effects of low-dose APL (NBI-5788) are currently undergoing safety evaluation.

T-cell vaccination strategies attempt to eliminate pathogenic T cells through the enhancement of regulatory immune responses to autoreactive T cells. This approach requires the isolation of autoreactive T-cell clones from the individual patient's blood or CSF, and subcutaneous reinjection in the form of an immunizing vaccine. Pilot trials of T-cell vaccination with autologous MBP-specific T cells from peripheral blood, in 28 RRMS and 26 SPMS patients, demonstrated a modest reduction in posttreatment relapse rate (Medaer *et al.*, 1995; Zhang *et al.*, 2002). In this study, the frequency of gadolinium-enhancing lesions was largely unchanged posttreatment. A second pilot trial using autologous MBP and MOG-reactive T-cell vaccines in 20 RRMS nonresponders demonstrated a significant reduction in relapse rate ( $p = 0.026$ ) as well as gadolinium enhancing and T2 lesion load (Achiron *et al.*, 2004). In both studies, no serious adverse events were noted. In a small study utilizing myelin-reactive CD4+ T cells derived from autologous CSF, no adverse effects were observed in any of the five treated patients (Van der Aa *et al.*, 2003b). Further larger phase II trials using T-cell vaccination are planned.

TCR vaccination strategies target TCR sequences believed to be critical in the immunopathogenesis of MS. In MS, V $\beta$  5.2/5.3+ has been identified as a dominant TCR variable region sequence involved in MBP T-cell reactivity (Kotzin *et al.*, 1991; Lozeron *et al.*, 1998; Oksenberg *et al.*, 1993). TCR vaccines are thought to exert their effects by enhancing the function of regulatory T-cell populations recognizing TCR determinants (Vandenbark, 2005; Vandenbark *et al.*, 1996). TCR peptides derived from the V $\beta$  5.2 region of the TCR have been used as a vaccine in MS patients. In a double-blind pilot study, 23 patients were treated with weekly to monthly injections of the peptide. All patients carried the HLADRB1\*1501 allele. Enhanced T-cell responses to the immunizing peptide correlated with clinical improvement (Bourdette *et al.*, 1994; Vandenbark *et al.*, 1996). T-cell responses to MBP trended downward in responders. No major adverse events were observed in treated patients. ATM-027 is an antibody specifically targeting the V $\beta$  5.2/5.3 sequence of TCR. Results from a multicenter phase II study in 47 MS patients treated with a run-in regimen of ATM-027 monthly for 6 months, showed no significant reduction in new gadolinium MRI

lesions posttreatment despite a significant reduction in V $\beta$  5.2/5.3+ T cells (Killestein *et al.*, 2002; Olsson *et al.*, 2002). MS relapses occurred in three treated patients, however no other adverse events directly related to drug were observed. These negative results suggest that there is considerable variability in V $\beta$  profiling in individual MS patients. An alternative explanation is that by the time the disease presents clinically, epitope spreading has occurred, negating the use of a single V $\beta$ -depleting agent.

CD4 is a cell-surface marker of Th cells. In a randomized phase II double-blind trial, an anti-CD4 antibody (cM-T412) was administered intravenously to 35 RRMS and SPMS patients (van Oosten *et al.*, 1997). Administration of the antibody resulted in a rapid and sustained reduction in circulating CD4+ T cells. Infusion-related side effects including nausea, fever, and tachycardia were limited to 24-h postinfusion. After 9 months, treated patients demonstrated an approximately 40% reduction in relapse rate compared to placebo controls, however there was no significant change in the number of gadolinium-enhancing lesions on MRI. Although anti-CD4 therapy was effective in reducing relapse rate, lack of efficacy on primary MRI measures has led to questions regarding the effectiveness in MS.

The Jak/STAT family of transducer/transcription-activating factors plays a critical role in the signaling of many cytokine receptors. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors such as atorvastatin has been shown to exert its protective effects in EAE through the induction of STAT6 phosphorylation and secretion of Th2 cytokines, with concomitant inhibition of STAT4 phosphorylation and secretion of Th1 cytokines (Youssef *et al.*, 2002). A pilot clinical trial of simvastatin in MS showed positive results and a larger trial of Atorvastatin is under way.

Inhibition of molecular pathways involved in T-cell migration has been effective in reducing MS relapses (Miller *et al.*, 2003), however clinical trials with an  $\alpha$ 4-integrin antibody led to several cases of progressive multifocal leukoencephalopathy, resulting in the reevaluation of the role of such drugs in MS therapeutics (Kleinschmidt-Demasters and Tyler, 2005; Langer-Gould *et al.*, 2005; Van Assche *et al.*, 2005).

#### IV. Conclusions

In conclusion, we have summarized the evidence for the central role of CD4+ T cells in the pathogenesis of MS, which represents the work of many teams of investigators over many years. Although much progress has been made in the field, which has led to important therapeutic advances, several questions remain unanswered, including the nature of the initiating T-cell responses and

the mechanisms of propagation of the disease within the CNS. The field of T-cell biology remains an integral part of the MS question, and further advances will undoubtedly lead to improved treatment strategies for patients.

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