Role of CD4+ T Cells in the Pathophysiology of Multiple Sclerosis

Fumitaka Sato[1,2], Seiichi Omura[1,2], S.L. Jaffe[3], Ikuo Tsunoda[1,2,3]

[1]Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, Shreveport, LA, USA; [2]Center for Molecular and Tumor Virology, Louisiana State University Health Sciences Center, Shreveport, LA, USA; [3]Department of Neurology, Louisiana State University Health Sciences Center, Shreveport, LA, USA

SUMMARY

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Although the precise etiology of MS remains unclear, CD4+ T cells have been proposed to play not only effector but also regulatory roles in MS. CD4+ T cells can be divided into four subsets: pro-inflammatory helper T (Th) 1 and Th17 cells, anti-inflammatory Th2 cells and regulatory T cells (Tregs). The roles of CD4+ T cells in MS have been clarified by either "loss-of-function" or "gain-of-function" methods, which have been conducted mainly in autoimmune and viral models of MS: experimental autoimmune encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus (TMEV) infection, respectively. Observations in MS patients were consistent with the mechanisms found in the MS models, that is, increased pro-inflammatory Th1 and Th17 activity is associated with disease exacerbation, while anti-inflammatory Th2 cells and Tregs appear to play a protective role.

TRADITIONAL CNS INFLAMMATION VERSUS "NEUROINFLAMMATION"

MS is an inflammatory demyelinating disease of the central nervous system (CNS) (Correale & Fiol, 2009; Jaffe, Glabus, Kelley, & Minagar, 2003; Kira, 2008). Immune cells, particularly T and B cells, have been implicated in the pathogenesis of MS (Johnson, Suidan, McDole, & Pirko, 2007; Sato, Omura, Martinez, & Tsunoda, 2011). Based on the expression of CD4 or CD8 antigens, T cells can be divided into two subpopulations: CD4+ and CD8+ T cells (Rotteveel et al., 1988). CD4+ and CD8+ T cells recognize extracellular and intracellular antigens presented on major histocompatibility complex (MHC) class II and I molecules, respectively (Table 1). CD4+ T cells help both cellular and humoral immunity, while CD8+ T cells function as cytotoxic T lymphocytes (CTLs) (Table 1). Most tissue cell types have been shown to express MHC class I molecules on their surface, while MHC class II molecules are expressed mainly on antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages (Table 2). On the other hand, in the resting CNS, the four major parenchymal neuronal cells (neurons, oligodendrocytes, astrocytes, and microglia) have been shown to express neither MHC class I nor MHC class II molecules. Upon

TABLE 1   T cells and MHC[a] restriction

Subpopulation	MHC restriction	Recognized antigen	Function
CD4+ T cell	MHC class II molecule	Extracellular antigen	Helper
CD8+ T cell	MHC class I molecule	Intracellular antigen	Cytotoxic

[a] *MHC, major histocompatibility complex.*

TABLE 2   MHC[a] expression on CNS[b] and peripheral cell types

	MHC class I molecule		MHC class II molecule	
	Resting	Activated	Resting	Activated
Neuron	−	−	−	−
Oligodendrocyte	−	+	−	−
Astrocyte	−	+	−	+
Microglia	−	+	−	+
Peripheral general cell	+	+	−	−
Peripheral APC[c]	+	+	+	+

[a] *MHC, major histocompatibility complex.*
[b] *CNS, central nervous system.*
[c] *APC, antigen-presenting cells.*

activation, however, astrocytes and microglia can express both MHC class I and II molecules, and oligodendrocytes can express MHC class I molecules but not MHC class II molecules, while neurons do not express MHC class I or II molecules under any conditions (Joly & Oldstone, 1992; Pender, 1995; Suzumura, Lavi, Weiss, & Silberberg, 1986).

Collectively, activation of glial cells is labeled "gliosis", while activation of astrocytes or microglia is termed "astrogliosis (or astrocytosis)" or "microgliosis", respectively (note: the term "proliferation" is not accurate for CNS parenchymal cells because neurons are postmitotic cells and even glial cells cannot increase their numbers mitotically). After activation, microglia and astrocytes change their morphology and express other molecules. These processes can be visualized histologically, for example, microglia activation by lectin cytochemistry, and astrocyte activation by immunohistochemistry against glial fibrillary acidic protein (GFAP) (Tsunoda, McCright, Kuang, Zurbriggen, & Fujinami, 1997). Activated astrocytes and microglia can also express immune-related molecules, such as pro-inflammatory cytokines including interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α, but not cytokines that are typically produced by lymphocytes, such as interferon (IFN)-γ or IL-2. Thus, the term "neuroinflammation" has often been used to describe not only conventional inflammatory CNS diseases but also several CNS neurological diseases traditionally categorized as noninflammatory, such as Parkinson's disease (Chen & Tansey, 2011), Alzheimer's disease, and stroke. In these latter diseases, lesions contain little or no extravasation of cellular or humoral components from blood vessels: (1) no parenchymal lymphocyte infiltration and (2) no blood–brain barrier (BBB) breakdown, while activated microglia and astrocytes have been shown to be present in or adjacent to lesions. For example, in Alzheimer's disease, the terms "chronic inflammation" and "vascular inflammation" have been used in the description of this condition, when immune-related molecules, such as adhesion molecules and pro-inflammatory cytokines, are upregulated on blood vessels, microglia, and astrocytes without extravasation of leukocytes from the blood vessels (Grammas, 2011). It should be noted that demyelination is not observed as a major neuroimmunopathological finding in the above "neuroinflammatory diseases."

Pathologically, the term "inflammation" is defined as the condition occurring when the tissue manifests rubor (redness), calor (heat), tumor (swelling), and dolor (pain), all of which are mainly caused by extravasation of blood cells and serum components associated with vasodilatation (Kumar, Abbas, Fausto, & Aster, 2009). As noted above, however, the term "inflammation" is often used in describing both systemic and CNS diseases, such as atherosclerosis, in which extravasation of leukocytes does not occur, but where there is upregulation of immune-related molecules. This more vague usage of the term "inflammation" has drawn attention

to the role of immune-related molecules in diseases that traditionally were defined as noninflammatory. However, this can easily confuse these diseases with true "inflammatory diseases," in which immune cells extravasated from the blood vessels to the parenchyma play a major role. Thus, in this chapter, we use the term "inflammation" only for conditions, where blood leukocytes extravasate from the blood vessels. In addition, to avoid confusion between "neuroinflammation" composed mainly of astrogliosis or microgliosis versus traditional inflammation, in which extravasation of leukocytes from the circulation to the CNS parenchyma occurs, we will use the term "gliosis" for the former and not the term "neuroinflammation" in the chapter sections which follow.

Although activation of astrocytes or microglia alone is not sufficient to induce demyelination, activation of microglia may be associated with triggering subsequent inflammatory demyelination. In early lesions of MS and its animal models, microglial nodules (small area of microgliosis) have been observed (Sato, Tanaka, Hasanovic, & Tsunoda, 2011; Singh et al., 2013; Tsunoda, Tanaka, Saijoh, & Fujinami, 2007). Interestingly, these early lesions do not contain demyelination, but often demonstrate axonal degeneration and/or oligodendrocyte apoptosis. Thus, while microgliosis can precede demyelination, full-blown demyelination has been shown to require recruitment of leukocytes from the systemic circulation, particularly CD4^{+} T cells.

ETIOLOGY OF MS

The clinical course of MS is classified into four forms (Lublin & Reingold, 1996; Tsunoda, Kuang, Theil, & Fujinami, 2000): relapsing–remitting (RR), primary progressive (PP), secondary progressive (SP), and progressive–relapsing (PR). RRMS is defined by disease attacks ("relapses") with full recovery ("remission") or with minimal sequelae (Sospedra & Martin, 2005). The majority of MS patients (85–90%) develop RRMS initially followed by SPMS, characterized by progressive neurological deficits (Ebers, 2005). PPMS (10–15% of patients) progresses continuously from the onset without remission (Noseworthy, Lucchinetti, Rodriguez, & Weinshenker, 2000). PRMS is a progressive disease from the onset with acute relapses with or without full recovery; periods between relapses are characterized by continuous progression (Martinez et al., 2013).

Although the precise pathomechanism of MS remains unclear, the etiology of MS has been associated with genetics, autoimmunity, and environmental factors, particularly viral infections (Sato, Omura, et al., 2011). Clinical studies have shown that genetic factors contribute to the pathogenesis of MS (Lynch et al., 1991; Pugliatti, Sotgiu, & Rosati, 2002; Schwendimann & Alekseeva, 2007; Seboun et al., 1989). Some haplotypes

of human leukocyte antigen (HLA), such as HLA-DRB1*1501, have been positively correlated with MS development (Dyment, Ebers, & Sadovnick, 2004). Genes, including those for IL-2 receptor α chain (CD25), IL-7 receptor α chain (CD127), and TNF receptors may influence the risk of developing MS (Oksenberg, Baranzini, Barcellos, & Hauser, 2001; Oksenberg, Baranzini, Sawcer, & Hauser, 2008; Rose, 2009). Genes for the vitamin D receptor (Fukazawa et al., 1999) and estrogen receptor (Niino, Kikuchi, Fukazawa, Yabe, & Tashiro, 2000) have also been linked to MS susceptibility. However, individuals who have the same genetic background do not always develop MS; concordance of MS was 30% among monozygotic twins, while their disease incidence was 10-fold greater than that seen in dizygotic twins (Ebers et al., 1986).

The autoimmune theory of MS is based on the detection of myelin-specific T cells and antibodies in MS patients (Burns, Bartholomew, & Lobo, 1999). Histologically, infiltration of immune cells, including CD4$^+$ and CD8$^+$ T cells, has been observed in active MS lesions (Hafler & Weiner, 1987). Clinically, immunomodulatory drugs, such as IFN-β, glatiramer acetate, and anti-very late antigen-4 antibody (Tysabri$^®$ (natalizumab)), have been shown to suppress the disease activity of RRMS patients (Minagar et al., 2003). Unlike other autoimmune diseases, however, neither autoantigen-specific T cell nor antibody responses can be used as biomarkers for diagnosis or prognosis of MS. In addition, although many autoimmune diseases have been shown to accompany primary immunodeficiency diseases (PIDs) (e.g., increased lupus-like diseases in complement deficiencies), MS has not been associated with PIDs (Al-Herz et al., 2011).

Environmental factors, particularly viral infections, have also been associated with MS pathogenesis (Deuschle, Bode, Heuser, Schmider, & Ludwig, 1998; Haase et al., 2001). Clinically, several viruses, such as herpes simplex virus, human herpesvirus 6, and coronavirus, have been isolated from MS patients (Murray, Brown, Brian, & Cabirac, 1992; Opsahl & Kennedy, 2005; Sanders et al., 1996). In addition, higher immune responses against certain viruses, such as Epstein-Barr virus and measles virus, have been reported in MS patients compared with healthy controls (Chiodi, Sunqvist, Link, & Norrby, 1987; Christensen, 2006; Lunemann et al., 2008). However, neither single virus nor virus-specific immune response has been uniformly detected in all MS patients.

Experimentally, two animal models of MS, experimental autoimmune encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus (TMEV) infection, have been most widely used as autoimmune and viral models of MS, respectively (Bahk, Kappel, Rasmussen, & Kim, 1997; Clatch, Melvold, Miller, & Lipton, 1985; Rodriguez, Leibowitz, & David, 1986; Tsunoda & Fujinami, 1996). These two models resemble MS both clinically and histologically (Sato et al., 2013). Most EAE models have been shown to be mediated by

CD4^{+} T cells that were induced by subcutaneous injection (sensitization) of CNS antigens, such as spinal cord homogenate, myelin basic protein (MBP), myelin proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG) (Martinez et al., 2013). EAE models can recapitulate some clinical aspects of MS: SJL/J mice develop relapsing–remitting EAE (RR-EAE) with PLP$_{139-151}$ or PLP$_{178-191}$ sensitization (Sobel, Tuohy, Lu, Laursen, & Lees, 1990; Tuohy, Lu, Sobel, Laursen, & Lees, 1989); C57BL/6 mice develop monophasic sustained EAE without complete remission, relapse, or progression with MOG$_{35-55}$ sensitization (Mendel, Kerlero de Rosbo, & Ben-Nun, 1995); and A.SW mice develop primary progressive EAE (PP-EAE) without remission (Tsunoda et al., 2000; Tsunoda, Libbey, Kuang, Terry, & Fujinami, 2005).

Intracerebral injection of TMEV into mice induces a biphasic disease. During the acute phase of TMEV infection, about 1 week post infection (p.i.), mice develop acute EAE but recover completely. During the chronic phase, approximately 1 month p.i., mice develop inflammatory demyelination with viral persistence in the spinal cord. The susceptibility to TMEV-induced demyelinating disease (TMEV-IDD) has been shown to differ among mouse strains. SJL/J mice are susceptible to TMEV-IDD and develop a chronic progressive paralysis without remission, while C57BL/6 mice can clear the virus and are resistant to TMEV-IDD (Kawai et al., 2014; Pullen, Park, Miller, Dal Canto, & Kim, 1995; Sato, Omura, et al., 2014). Diverse immune factors, including CD4^{+} and CD8^{+} T cells and antibodies, have been shown to play either pathogenic or protective roles in TMEV-IDD (Sato, Omura, et al., 2011).

PHYSIOLOGICAL AND PATHOLOGICAL ROLES OF CD4^{+} T CELL SUBSETS

While Mosmann et al. (1986) originally classified CD4^{+} T cells into two subsets: helper T (Th) 1 and Th2 cells, two more subsets of CD4^{+} T cells, Th17 cells and regulatory T cells (Tregs) (Note: regulatory T cell is commonly abbreviated as "Treg" instead of "Treg cell") have been identified (Bettelli, Oukka, & Kuchroo, 2007). Depending on the transcription factors regulated under distinct cytokine milieus, naïve CD4^{+} T cells are differentiated into the four subsets, each of which produces distinct cytokines (Sato, Omura, et al., 2011) (Table 3). IL-12 induces a transcription factor, T-box 21 (TBX21), in humans and T-box expressed in T cells (T-bet) in mice, which leads to differentiation into Th1 cells. Th1 cells produce large amounts of IFN-γ and IL-2, and mediate cellular immune responses, including delayed-type hypersensitivity (DTH) and clearance of intracellular pathogens. IL-4 enhances the expression of a transcription factor, GATA binding protein 3 (GATA3 (humans)/Gata3 (mice)), which promotes differentiation into Th2 cells. Th2 cells produce large amounts of

IL-4, IL-5, and IL-13, and help humoral immune responses, such as antibody production. The specific cytokines released from Th1 and Th2 cells can, respectively, suppress Th2 and Th1 cells.

Th17 cells express a transcription factor, retinoic-acid-receptor-related orphan receptor C (RORC) in humans and RORγt in mice, which promotes differentiation into Th17 cells. Tregs express CD25 and a transcription factor, forkhead box P3 (FOXP3), in humans and Foxp3 in mice, which promotes differentiation into Tregs (Baecher-Allan, Viglietta, & Hafler, 2004). The balance between the transforming growth factor (TGF)-β and IL-6 acts to induce either RORC/RORγt or FOXP3/Foxp3. Th17 cells produce IL-17A, IL-17F, IL-21, and IL-22, and have been shown to play a role in antibacterial and fungal immunity as well as immune-mediated tissue damage (immunopathology). The cytokines released from Th17 cells can suppress Th1 cells, while Th1- and Th2-associated cytokines, such as IFN-γ and IL-4, can inhibit the differentiation of Th17 cells. Tregs produce TGF-β and IL-10 and suppress the other Th cell functions, particularly autoimmunity and anti-tumor immunity; the suppression of autoimmunity is protective but that of anti-tumor immunity is detrimental in hosts (Matsui et al., 2010).

The immunoregulatory axis composed of the four subsets of CD4+ T cells has been proposed to play either a pathogenic or protective role in MS. How do CD4+ T cells contribute to either pathogenesis or protection

TABLE 3 CD4+ T cell subsets

Subset	Transcription factor	Cytokine	Physiological function	Detrimental role
Th1[a]	TBX21/T-bet[b]	IFN-γ[c], IL-2[d]	Intracellular pathogen clearance	DTH[e] responses
Th2	GATA3/Gata3[f]	IL-4, 5, 13	Help antibody production in extracellular pathogen clearance	Help autoantibody production
Th17	RORC/RORγt[g]	IL-17A, 17F, 21, 22	Antibacterial and fungal immunity	Immunopathology
Treg[h]	FOXP3/Foxp3[i]	IL-10, TGF-β[j]	Suppress autoimmunity	Suppress tumor and antiviral immunities

[a] Th1, helper T 1.
[b] TBX21 (humans)/T-bet (mice), T-box 21/T-box expressed in T cells.
[c] IFN-γ, interferon-γ.
[d] IL-2, interleukin-2.
[e] DTH, delayed-type hypersensitivity.
[f] GATA3, GATA binding protein 3.
[g] RORC/RORγt, retinoic-acid-receptor-related orphan receptor C (humans)/γt (mice).
[h] Treg, regulatory T.
[i] FOXP3 (humans)/Foxp3 (mice), forkhead box P3.
[j] TGF-β, transforming growth factor-β.

in MS? In the CNS, CD4+ T cells can recognize myelin antigens presented by MHC class II molecules on activated microglia and astrocytes or on CNS-infiltrating immune cells, but not on oligodendrocytes or neurons (Tables 1 and 2). After antigen recognition, CD4+ T cells can produce cytokines: Th1 and Th17 cells produce pro-inflammatory cytokines, while Th2 cells and Tregs produce anti-inflammatory cytokines. Theoretically, the released pro-inflammatory cytokines further recruit inflammatory cells to the CNS, which enhance inflammation and damage oligodendrocytes and axons, resulting in demyelination and axonal degeneration. Pro-inflammatory cytokines have been shown to directly or indirectly induce apoptosis of oligodendrocytes in vitro (Selmaj, Raine, Farooq, Norton, & Brosnan, 1991; Vartanian, Li, Zhao, & Stefansson, 1995). In turn, apoptotic oligodendrocytes and degenerated axons can further activate microglia and macrophages, which can exacerbate immunopathology. Although the anti-inflammatory Th2- and Treg-associated cytokines that are produced from CD4+ T cells have been proposed to suppress immunopathology (Weber, Hohlfeld, & Zamvil, 2007), these cytokines, particularly Th2 cytokines, can also enhance autoantibody production (Ulusoy et al., 2012), contributing to tissue damage. In most demyelinating model systems, CNS damage is likely caused by indirect attack by soluble molecules, but not direct cellular contact ("cytotoxicity") by CD4+ T cells (Sato, Tanaka, et al., 2011). Here, in theory, any inflammation in the CNS can induce demyelination. Interestingly, however, only limited numbers of antigens and viruses (i.e., myelin antigens and a few viruses, such as TMEV) have been known to induce demyelination. Clinically, it is uncommon for CNS microbial inflammatory diseases to result in demyelination with a few exceptions, including acute disseminated EAE and related diseases. Experimentally, it is also rare that nonmyelin antigens induce demyelination in the CNS (Derfuss et al., 2009; Mathey et al., 2007).

CLASSICAL Th1/Th2 IMMUNOREGULATORY AXIS IN MS AND ITS ANIMAL MODELS

Role of Th1 cells

The Th1/Th2 immunoregulatory axis, in which pro-inflammatory Th1 cells and anti-inflammatory Th2 cells oppose each other, has been proposed to regulate the pathogenesis of MS (Martinez et al., 2013). This theory has been supported by clinical studies in MS, where Th1 immune responses were positively associated with the disease activity in MS patients (Hofman et al., 1986; Hofman, Hinton, Baemayr, Weil, & Merrill, 1991). Merrill et al. (1989) reported that CD4+ T cell lines derived from the cerebrospinal fluid (CSF) of MS patients produced

larger amounts of IFN-γ and IL-2. Mechanistically, although "exacerbation" of MS by IFN-γ administration initially seemed to support this theory (Panitch, Hirsch, Haley, & Johnson, 1987; Panitch, Hirsch, Schindler, & Johnson, 1987), the results of this trial are now controversial for several reasons.

Similar descriptive findings in MS patients have been observed in an autoimmune model of MS, EAE. For example, CD4+ T cells isolated from the CNS and periphery of EAE mice have been shown to preferentially produce Th1 cytokines, including IFN-γ and IL-2 (Ando, Clayton, Kono, Urban, & Sercarz, 1989; Merrill et al., 1992). Mechanistically, the pathogenic role of Th1 cells in EAE has been investigated by mainly "loss-of-function" approaches using gene knockout (KO) mice or blockade of Th1 cytokines by neutralizing antibodies. Constantinescu et al. (2001) demonstrated that the prophylactic treatment with anti-IL-12 neutralizing monoclonal antibody (mAb) rendered susceptible SJL/J mice resistant to EAE induced with mouse spinal cord homogenate. In MOG-induced EAE, T-bet-deficient mice developed less severe EAE with lower amounts of IFN-γ production, compared with wild-type mice (Bettelli et al., 2004). Adoptive transfer of in vitro activated and expanded T cells into naïve mice has been used to test the encephalitogenicity of the transferred T cell population (passive EAE); myelin antigen-specific Th1 cells have been shown to induce EAE upon such transfer (Ando et al., 1989; Baron, Madri, Ruddle, Hashim, & Janeway, 1993; Merrill et al., 1992).

In the viral model of MS, TMEV-IDD, there have been inconsistent findings on the role of Th1 cells. During the early chronic phase of TMEV infection, DTH responses to TMEV, which are mainly mediated by Th1 cells, have been associated with demyelination (Miller et al., 1990). During the late chronic phase of TMEV infection, myelin-specific Th1 cells induced by "epitope (or determinant) spreading" (from viral epitopes to myelin epitopes) have been proposed to exacerbate the demyelinating disease (Miller et al., 1997), although epitope spreading was not detected by other groups (Tsunoda & Fujinami, 2005). Mechanistically, although Inoue et al. (1998) demonstrated that injection of anti-IL-12 neutralizing mAb during the early chronic phase of TMEV infection attenuated TMEV-IDD with decreased production of IFN-γ, the effect of IFN-γ modulation on TMEV-IDD differed depending on the experimental settings (Tsunoda & Fujinami, 1999).

Role of Th2 cells

Th2 cells may play a protective role in MS, as Th2 immune responses have been shown to increase during remission in RRMS (Araki et al., 2003; Clerici et al., 2001). Decreased disease progression and exacerbation of MS during pregnancy have been associated with Th2-biased

immune responses (Al-Shammri et al., 2004), although the exact mechanism remains unclear. Suppression of MS disease activities by immunomodulatory drugs, such as glatiramer acetate, has also been associated with enhanced Th2 immune responses (Weber et al., 2007). Experimentally, Th2 cells have been shown to regulate EAE and TMEV-IDD. In EAE induced with mouse spinal cord homogenate, injection of anti-IL-4 neutralizing mAb during the induction phase rendered resistant BALB/c mice susceptible to EAE (Constantinescu et al., 2001). The adoptive transfer of PLP-specific Th2 cell clones at the time of sensitization or disease onset prevented EAE in mice sensitized with PLP (Kuchroo et al., 1995). While T cell immunoglobulin mucindomain containing (TIM)2 has been shown to be preferentially expressed on the surface of Th2 cells and to negatively regulate Th2 immune responses, blockade of TIM-2/TIM-2 ligand interaction by administration of soluble TIM-2 fusion protein delayed the onset and decreased the severity of PLP-induced EAE by enhancing Th2 immune responses (Chakravarti et al., 2005). In TMEV-IDD, Th2 immune responses have also been demonstrated to suppress inflammatory demyelination in the CNS. Hill et al. (1998) demonstrated that during the early chronic phase of TMEV infection, infected mice treated with IL-4 developed less severe inflammatory demyelination compared with controls. Thus, the findings in EAE and TMEV-IDD suggest that Th1 cells could contribute to the pathogenesis of MS, while Th2 cells may play a protective role (Table 3).

Unconventional role of Th1 and Th2 cells

Depending on the disease stage and models, Th1 and Th2 cells have been shown to play both a beneficial and detrimental role in animal models of MS (Martinez et al., 2013). In EAE, Ferber et al. (1996) demonstrated that IFN-γ-deficient B10.PL mice developed more severe MBP-induced EAE, compared with susceptible wild-type B10.PL mice. Tran et al. (2000) demonstrated that wild-type BALB/c mice were resistant to MBP-induced EAE, while IFN-γ- or IFN-γ receptor-deficient BALB/c mice developed inflammatory demyelination in the CNS. Moreover, Willenborg et al. (1996, 1999) demonstrated that a lack of IFN-γ receptor rendered an EAE-resistant 129/Sv mouse strain susceptible to MOG-induced EAE. In TMEV-IDD, during the acute phase of TMEV infection, Th1 cells seemed to contribute to viral clearance, since IFN-γ neutralization with anti-IFN-γ mAb significantly accelerated the onset of TMEV-IDD (Pullen, Miller, DalCanto, Van der Meide, & Kim, 1994).

Unlike conventional monophasic EAE and RR-EAE models, the disease course of some progressive EAE models has been associated with Th2 immune responses. Lafaille et al. (1997) demonstrated that the adoptive transfer of MBP-specific Th2 cells caused progressive

EAE in immunocompromised recombination-activating gene-1 (RAG-1)-deficient mice and in αβ T cell-deficient mice, but not in wild-type mice. Th2 immune responses may also exacerbate EAE by enhancing pathogenic autoantibody production against MOG. Tsunoda et al. (2000) demonstrated that MOG-sensitized A.SW mice mounted high anti-MOG antibody responses with Th2-biased immune responses and extensive demyelination with immunoglobulin (Ig) deposition in the CNS, resulting in progressive and fatal EAE. Furthermore, in MOG-induced EAE, apoptotic cell injection in SJL/J mice altered the disease course from RR-EAE to secondary progressive EAE (SP-EAE) with the enhancement of anti-MOG antibody production and Th2-biased immune responses (Tsunoda, Libbey, et al., 2005). Similarly, in an SP-EAE model induced by MOG sensitization with ultraviolet (UV) irradiation, SJL/J mice with SP-EAE developed more severe demyelinating disease with higher anti-MOG antibody responses and lower Th1 immune responses, compared with control SJL/J mice with RR-EAE, which were not irradiated with UV (Tsunoda, Kuang, Igenge, & Fujinami, 2005). Thus, in some cases of MS, Th1 cells may play a protective role, while Th2 cells may be associated with disease progression.

NOVEL Th17/TREG IMMUNOREGULATORY AXIS IN MS AND ITS ANIMAL MODELS

Role of Th17 cells

The Th17/Treg paradigm was identified as a novel immunoregulatory axis composed of pro-inflammatory Th17 cells and anti-inflammatory Tregs. Th17 cells can promote inflammation through pro-inflammatory cytokine and chemokine expression and neutrophils recruitment (Martinez et al., 2012). Although the precise role of Th17 cells in immune-mediated tissue damage remains unclear, there have been reports investigating the effector mechanism of Th17 cells in MS. In patients with RRMS, the number of Th17 cells in the CSF was higher during relapse than during remission (Brucklacher-Waldert, Stuerner, Kolster, Wolthausen, & Tolosa, 2009), suggesting that Th17 immune responses were associated with disease activity in MS patients. Increased IL-17 messenger RNA and protein levels have been detected in the CSF, peripheral blood mononuclear cells (PBMCs), and brain lesions of MS patients, particularly during relapses (Lock et al., 2002; Matusevicius et al., 1999; Tzartos et al., 2008). In addition, nuclear receptor subfamily 4, group A, member 2 (NR4A2), which controls Th17 cell function via IL-21 signaling, has been shown to increase in T cells from PBMCs of MS patients (Doi et al., 2008; Raveney, Oki, & Yamamura, 2013).

The increased frequency of Th17 cells in EAE has supported the effector role of Th17 cells in MS. Hofstetter et al. (2005) first demonstrated that IL-17 production was detected in splenic T cells from EAE mice sensitized with MOG, but not in those from naïve mice, following in vitro stimulation with MOG. IL-17 neutralization with IL-17-receptor-Fc-hybrid protein or anti-IL-17 mAb partially ameliorated EAE. Similarly, Komiyama et al. (2006) reported that, in MOG-induced EAE, IL-17-deficient C57BL/6 mice showed delayed onset and decreased severity of EAE, compared with wild-type C57BL/6 mice. In addition, upon passive transfer into naïve wild-type mice, CD4⁺ T cells from MOG-sensitized wild-type C57BL/6 mice induced EAE in the recipient mice with a higher incidence than those from MOG-sensitized IL-17-deficient C57BL/6 mice. Raveney et al. (2013) demonstrated that injection of NR4A2-specific siRNA before the onset of MOG-induced EAE reduced Th17-related molecules, including expression of IL-23 receptor and IL-21, resulting in less severe clinical signs. Bettelli et al. (2006) demonstrated that TGF-β-transgenic (Tg) C57BL/6 mice with enhanced TGF-β production from T cells had a higher frequency of Th17 cells and developed more severe MOG-induced EAE than wild-type C57BL/6 mice.

In TMEV-IDD, Hou et al. (2009) found that the percentage/number of Th17 cells among CNS mononuclear cells (MNCs) were higher in TMEV-susceptible SJL/J mice than in TMEV-resistant C57BL/6 mice, suggesting a detrimental role for Th17 cells. CNS-infiltrating MNCs produced more IL-17 in TMEV-infected SJL/J mice than in TMEV-infected C57BL/6 mice, following in vitro TMEV antigen stimulation. Anti-IL-17 neutralizing mAb injection into TMEV-infected SJL/J mice inhibited viral persistence in the CNS and enhanced the function of CTLs against TMEV, resulting in reduced incidence and severity of TMEV-IDD. While IL-6 has been known to promote Th17 cell differentiation as well as inflammation, TMEV-infected IL-6-Tg C57BL/6 mice, which produce human IL-6 under the control of MHC class I promoter, showed clinical signs with findings of increased viral loads and Th17 immune responses to TMEV in the CNS (Hou, Jin, Kang, & Kim, 2014). Thus, Th17 cells could play a pathogenic role in MS (Table 3).

Role of Tregs

Tregs have been shown to contribute to the maintenance of immunologic tolerance by suppressing auto-reactive lymphocytes due to the production of anti-inflammatory cytokines such as IL-10 and TGF-β. FOXP3/Foxp3-deficient humans and mice have been shown to develop autoimmune diseases: immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, and scurfy mice, respectively (Sakaguchi, Yamaguchi, Nomura, & Ono, 2008; Ziegler, 2006). Interestingly, however, it should be noted that MS-like CNS disease was neither reported in

IPEX syndrome nor in scurfy mice. Clinically, in PBMCs of MS patients, Tregs were present at a lower number and had functional defects (Oh et al., 2009; Viglietta, Baecher-Allan, Weiner, & Hafler, 2004). Experimentally, the blockade of Tregs by anti-CD25 mAb has been shown to exacerbate EAE. Montero et al. (2004) demonstrated that anti-CD25 mAb injection prior to MOG sensitization exacerbated EAE with findings of increased MOG-specific Th1 and antibody responses. Reddy et al. (2004) also demonstrated that the blockade of Tregs by anti-CD25 mAb rendered resistant B10.S mice susceptible to PLP-induced EAE. In this system, the anti-CD25 mAb treatment increased the incidence of EAE and enhanced inflammation in the CNS with higher IFN-γ and lower IL-10 production from CD4$^+$ T cells, compared with the control antibody treatment. However, caution should be used in interpreting the data from anti-CD25 antibody studies, since CD25 is expressed on not only Tregs but also activated T cells. Clinically, treatment with anti-CD25 antibody (Zenapax$^®$ (daclizumab)) has been reported to have therapeutic effects on MS by reducing the population of activated T cells, although the treatment reduced the number/function of Tregs (Oh et al., 2009; Pfender & Martin, 2014). Experimentally, treatment with anti-CD25 mAb also inhibited a passive EAE model, where the donor encephalitogenic T cell population was stimulated with MBP in the presence of anti-CD25 mAb in vitro and transferred into naïve recipient animals (Hayosh & Swanborg, 1987). These results showed that anti-CD25 mAb treatment could suppress immune effector cell functions in both humans and animals. Furthermore, during the T cell development in the thymus, CD25 expression has been widely used as a developmental marker for murine CD4$^-$CD8$^-$ double negative (DN)2 and DN3 cells (Godfrey, Kennedy, Suda, & Zlotnik, 1993; Roifman, 2005; Rothenberg & Yui, 2008). However, the potential effect of anti-CD25 antibody treatment on T cell development has never been discussed in the above studies.

On the other hand, Kohm et al. (2002) demonstrated that recipient mice transferred intravenously with Tregs 3 days before EAE induction showed significantly less clinical signs, compared with the recipient mice transferred with non-Treg cells. In addition, the coinjection of MOG$_{35-55}$-specific T cells with Tregs suppressed the development of passive EAE in the recipient mice, while the transfer of MOG$_{35-55}$-specific T cells alone or with non-Treg cells induced severe EAE. Similarly, Selvaraj and Geiger (2008) demonstrated that induced Tregs (iTregs), which were induced in the presence of TGF-β in vitro, inhibited MOG-induced EAE, when the iTregs were given to mice at the onset of EAE. After in vitro MOG stimulation, MNCs from EAE mice injected with iTregs had higher levels of MOG-specific IL-10 production but lower levels of IFN-γ and IL-17 production, compared with those from EAE mice injected with PBS and non-Treg cells. Although the precise immuno-suppressive mechanism of Tregs remains unclear, IL-10 production from Tregs has been shown to be critical for

immunosuppression in some EAE models, as Tregs from IL-10-deficient mice failed to prevent EAE in recipient mice (Selvaraj & Geiger, 2008; Yu et al., 2005; Zhang et al., 2004).

In contrast, in TMEV-IDD, a detrimental role for Tregs has been suggested (Richards et al., 2011). After TMEV infection, the number of Tregs in the CNS rapidly increased in susceptible SJL/J mice, but not in resistant C57BL/6 mice. Blockade of Tregs by anti-CD25 mAb during the chronic phase of TMEV infection in SJL/J mice decreased the clinical signs of TMEV-IDD. On the other hand, Martinez, Karlsson, et al. (2014) demonstrated that Tregs can play not only detrimental but also protective roles in TMEV-IDD by administering iTregs to TMEV-infected SJL/J mice at different time points. When SJL/J mice were treated with iTregs on the same day as initiation of TMEV infection (iTreg-early), iTreg-early SJL/J mice showed more severe clinical signs with higher CNS viral loads during the acute phase of TMEV infection, compared with untreated control mice. Since iTreg-early SJL/J mice had less immune cell infiltration in the CNS during the acute phase of TMEV infection compared with control mice, iTregs appeared to suppress the CNS recruitment of antiviral immune cells, resulting in increased CNS viral loads in iTreg-early SJL/J mice. In contrast, when SJL/J mice were treated with iTregs during the late phase of TMEV infection (iTreg-late), iTreg-late SJL/J mice developed less CNS inflammatory demyelination and a greater amount of IL-10 production, compared with untreated control mice. Here, IL-10 was produced from multiple cell types, including CD4⁺ and CD8⁺ T cells, B cells, macrophages, and DCs, which was consistent with the findings that Tregs could induce IL-10 production from other cell types (Anghelina, Zhao, Trandem, & Perlman, 2009; Kearley, Barker, Robinson, & Lloyd, 2005). The contrasting effects of iTregs in TMEV infection suggested that Tregs could be beneficial by suppressing immunopathology, when CNS viral loads were low (iTreg-late) as seen with persistent viral infection, while Tregs could be detrimental by suppressing antiviral immunity, when viral replication is active (iTreg-early) as in acute viral infection. Thus, Tregs could play a beneficial or detrimental role depending on the disease stage (Table 3).

DO "GAIN-OF-FUNCTION" CHANGES AFFECT SUSCEPTIBILITY TO MS?

As described above, the roles of Th cell subsets have been investigated mainly by using a "loss-of-function" approach. Although these studies are informative and mimic human treatment with immunosuppressive therapy, "gain-of-function" mutations in the human immune system have been reported to alter immune responses, including Th cell subsets, and to increase susceptibility to several inflammatory diseases as

well as infections, for example, hemolytic uremic syndrome and chronic mucocutaneous candidiasis (Yamazaki et al., 2014). These reports raise the question whether a genetic enhancement of Th cell subsets could affect susceptibility to inflammatory demyelinating diseases, such as MS. We have established T-bet, Gata3, and RORγt-Tg mice that overexpress T-bet, Gata3, and RORγt in T cells and have Th1-, Th2-, and Th17-biased immune responses, respectively, on the C57BL/6 mouse background (Table 4) (Ishizaki et al., 2007; Yoh et al., 2003, 2012). We induced EAE or TMEV-IDD in both wild-type C57BL/6 littermates and Tg mice. In this system, wild-type C57BL/6 mice develop monophasic EAE with MOG$_{35-55}$ sensitization, but are resistant to TMEV-IDD. As expected, in EAE, Th17-biased RORγt-Tg mice developed more severe CNS demyelinating lesions, compared with wild-type C57BL/6 mice (Martinez, Sato, Omutra, et al., 2014). In TMEV infection, RORγt-Tg mice developed inflammatory demyelination in the CNS (Figure 1(C)), comparable to that of susceptible SJL/J mice (Figure 1(A)), while resistant C57BL/6 mice had no demyelination (Figure 1(B)) (Martinez, Sato, Kawai, et al., 2014). On the other hand, Th2-biased Gata3-Tg mice showed a delayed onset of MOG-induced EAE and less severe clinical signs, compared with wild-type C57BL/6 mice (Fernando et al., 2014), while Gata3-Tg mice remained

TABLE 4   Effect of "gain-of-function" intervention in Th[a] cell subsets on MS[b] models

Subset	Model system	EAE[c]	TMEV-IDD[d]	References
Th1	T-bet[e]-Tg[f] mice	No disease	No demyelination	Martinez, Sato, Omutra, et al. (2014)
Th2	Gata3[g]-Tg mice	Suppression	No effect	Fernando et al. (2014)
Th17	RORγt[h]-Tg mice	Exacerbation	Exacerbation	Martinez, Sato, Kawai, et al. (2014) and Martinez, Sato, Omutra, et al. (2014)
Treg	iTreg[i] injection	Suppression	Suppression[j]	Martinez, Karlsson, et al. (2014) and Selvaraj and Geiger (2008)

[a] Th, helper T.

[b] MS, multiple sclerosis.

[c] EAE, experimental autoimmune encephalomyelitis.

[d] TMEV-IDD, Theiler's murine encephalomyelitis virus-induced demyelinating disease.

[e] T-bet, T-box expressed in T cells.

[f] Tg, transgenic.

[g] GATA3, GATA binding protein 3.

[h] RORγt retinoic-acid-receptor-related orphan receptor γt.

[i] iTreg, induced regulatory T cell.

[j] iTreg injection suppressed TMEV-IDD during the chronic phase of TMEV infection, although iTreg injection exacerbated acute TMEV infection (see the text).
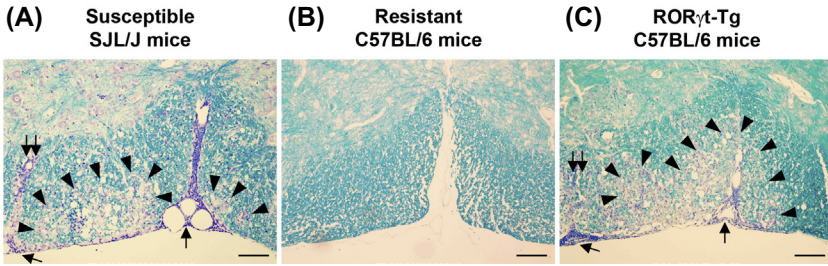
FIGURE 1 Chronic spinal cord pathology of mice infected with a Theiler's murine encephalomyelitis virus (TMEV). (A) Susceptible SJL/J mice developed demyelination (arrowheads) with meningitis (arrows) and perivascular cuffing (paired arrows) in the spinal cord. (B) Resistant C57BL/6 mice had no spinal cord pathology. (C) Retinoic-acid-receptor-related orphan receptor (ROR)γt-transgenic (Tg) C57BL/6 mice developed TMEV-induced demyelinating disease (TMEV-IDD) comparable to SJL/J mice. SJL/J, C57BL/6, and RORγt-Tg C57BL/6 mice were infected with TMEV intracerebrally. The spinal cords were harvested during the chronic phase, 2 months after TMEV infection. The sections were stained with Luxol fast blue for myelin visualization. Scale bars = 100 μm.

as resistant to TMEV-IDD as wild-type C57BL/6 mice (Sato, Fernando, et al., 2014). Interestingly, Th1-biased T-bet-Tg mice did neither develop EAE nor TMEV-IDD (Martinez, Sato, Omutra, et al., 2014; Sato, Fernando, et al., 2014). Thus, a genetic bias toward one Th cell subset could lead to a distinct effect on the susceptibility to inflammatory demyelinating diseases, depending on the etiology, autoimmune versus viral infection.

"T CELL EXHAUSTION" AS A PROTECTIVE MECHANISM AGAINST IMMUNOPATHOLOGY

"T cell exhaustion", that is, a loss of T cell function and number, has been demonstrated in chronic viral infections, such as with human immunodeficiency virus, hepatitis C virus, hepatitis B virus, and lymphocytic choriomeningitis virus (LCMV), as well as in patients with malignancies, such as melanoma and lung cancer (Speiser et al., 2014; Wherry, 2011). T cell exhaustion has been studied mainly in CD8+ T cells but has also been reported with CD4+ T cells (Yi, Cox, & Zajac, 2010). In this process, continuous high antigen stimulation has been proposed to cause the following changes: initially, virus-specific T cells can produce multiple cytokines, including IL-2, TNF-α, and IFN-γ, but then gradually lose this ability (Eikawa, Mizukami, & Udono, 2014). The T cells first lose the ability to produce IL-2 followed by TNF-α, and then later produce only IFN-γ or no cytokines (Yi et al., 2010). Proliferative ability as well as T cell repertoire number of virus-specific T cells have also been shown to decrease over time. Antiviral immune responses also decrease due to increased apoptosis

of antiviral T cells, leading to an increase in viral load. While activation marker expression on antiviral T cells, such as CD62L (L-selectin), CD122 (β-chain of IL-2 and IL-15 receptor), and CD127, is downregulated, the expression of inhibitory receptors on the T cells, including programmed cell death 1 (PD-1), TIM-3, lymphocyte-activated gene-3 (LAG-3), and cytotoxic T-lymphocyte antigen 4 (CTLA-4), is upregulated. In addition to the inhibitory receptors, anti-inflammatory cytokines, IL-10 and TGF-β, as well as Tregs can play a role in T cell exhaustion (Yi et al., 2010). Why do T cells develop this sequence of events that is detrimental to the host, leading to an increased viral load or cancer growth? One teleological idea regarding the reason that "exhaustion" evolved is that it limits immunopathology (Wherry, 2011).

The concept of "T cell exhaustion" has been questioned (Salek-Ardakani & Schoenberger, 2013; Speiser et al., 2014). For example, Hosking, Flynn, Botten, and Whitton (2013) showed that in mice immunized with LCMV, the same phenomenon that has been described as T cell exhaustion was observed to occur as rapidly as within 24 h after the second challenge with LCMV: a hierarchical loss of multiple cytokine responsiveness with an early termination of IFN-γ production, upregulation of inhibitory molecules, and a slight increase in T cell apoptosis. Although these T cells "appeared" exhausted, they did not lose antiviral CTL activity when the CTL responses were evaluated by in vivo CTL assays, and the T cells retained granzyme B upregulation. The retained cytolytic activity by CD8+ T cells had not been observed most likely because most CTL assays were conducted ex vivo (or in vitro), under which situation a CTL response does not occur often (Barber, Wherry, & Ahmed, 2003). This selective T cell suppression is called "split exhaustion", where T cells retain the beneficial antiviral CTL function with suppression of the potentially harmful production of IFN-γ which can induce immunopathology. This suggests that the phenomenon described as "T cell exhaustion" may be a physiological transition (functional specialization or "adaptation") of T cells to keep beneficial function while preventing immunopathology (Speiser et al., 2014).

Although one may argue that this "physiological" function of split exhaustion appears too good to be true, a similar "split functionality" has been observed in Tregs (Wherry, 2011). In most studies, Tregs have been shown to suppress immunopathology without suppression of antiviral immune responses and thus without an increase in viral load. As we described above, however, this beneficial outcome with Tregs can depend on the timing during the course of viral infection (Martinez, Karlsson, et al., 2014). While we found that the suppression of immunopathology in TMEV-IDD by iTregs was associated with IL-10, it is unclear whether iTregs can induce "split exhaustion" in CD8+ T cells either directly or indirectly, for example, by suppressing IL-2 production by Th1 cells (Yi et al., 2010).

PROTECTIVE ROLES OF PD-1 AND TIM-3 IN MS AND ITS ANIMAL MODELS

PD-1, an inhibitory receptor, can be induced upon activation of many types of immune cells, including T cells, B cells, natural killer cells, monocytes, and DCs (Zhao, Li, Leak, Chen, & Hu, 2014). PD-1 has two biding ligands: PD-L1 (programmed cell death 1 ligand 1, also called B7-H1) and PD-L2 (also called B7-H2), both of which are members of the B7 family of costimulatory molecules. While PD-L1 is constitutively expressed on T cells, B cells, macrophages, and DCs (Phares et al., 2009), PD-L2 expression is mainly restricted to APCs, including macrophages, DCs, and B1 B cells (Zhong, Tumang, Gao, Bai, & Rothstein, 2007). In humans, while the expression of PD-L1 was low or undetectable in the resting CNS tissues, the expression was induced particularly on astrocytes and microglia upon activation. Although PD-L2 expression is inconclusive in the CNS resident cells, the expression has been reported to be lower in brain endothelial cells from MS patients than in those from healthy controls. In addition, in MS lesions, while CD4+ T cells expressed PD-1, the majority of CD8+ T cells did not express PD-1. Thus, in MS lesions, the interaction between PD-1 and PD-L1/PD-L2 may be impaired, leading to exacerbation of immunopathology.

Experimentally, deficiency of PD-1 or blockade of PD-1/PD-L1 interaction has been shown to exacerbate animal models of MS. Salama et al. (2003) demonstrated that expression of PD-1 and PD-L1 progressively increased in the CNS after EAE induction with MOG and that blockade of PD-1 by anti-PD-1 mAb exacerbated EAE with an increased infiltration of CD4+ and CD8+ T cells into the CNS. Following in vitro MOG stimulation, IFN-γ production was higher in splenic MNCs from EAE mice treated with anti-PD-1 mAb than in those from untreated control mice. Furthermore, Carter et al. (2007) demonstrated that PD-1- or PD-L1-deficient mice, but not PD-L2-deficient mice, developed more severe MOG-induced EAE than wild-type mice with increased production of pro-inflammatory cytokines, including IFN-γ and IL-17 from MNCs isolated from draining lymph nodes. Similar to EAE, the levels of PD-1 and PD-L1 expression increased over the course of TMEV infection. In TMEV-IDD, blockade of PD-1/PD-L1 interaction enhanced proinflammatory cytokine production from CNS-infiltrating T cells, resulting in exacerbation of clinical signs without alteration of CNS viral loads (Duncan & Miller, 2011; Takizawa et al., 2014). Thus, engagement of PD-1 with PD-L1/PD-L2 may be associated with suppression of MS. However, the above experiments investigating the role of PD-1 and PD-L1/PD-L2 cannot rule out the effects on other immune cells in EAE and TMEV-IDD, because PD-1, PD-L1, and PD-L2 are expressed on many types of immune cells other than T cells.

Although TIM-3 has been described to be preferentially expressed on Th1 cells but not on Th2 or Th17 cells, TIM-3 is also expressed on activated CD8+ T cells, microglia, and DCs (Lee & Goverman, 2013). Interaction of TIM-3 with its ligand, galectin-9, has been shown to suppress Th1 cell functions (Rodriguez-Manzanet, DeKruyff, Kuchroo, & Umetsu, 2009). Clinically, TIM-3 expression was lower in T cell clones isolated from the CSF of MS patients than in those of healthy controls, where TIM-3 expression was defective on CD4+ T cells from MS patients (Hafler & Kuchroo, 2008; Koguchi et al., 2006). Experimentally, blockade of TIM-3/galectin-9 interaction using anti-TIM-3 antibody exacerbated PLP-induced EAE (Monney et al., 2002). In TMEV-IDD, Kaneyama et al. (2014) found that TIM-3 and galectin-9 expression increased over the disease course, and that mice injected with anti-TIM-3 neutralizing mAb developed more severe clinical signs compared with control mice. Thus, interaction of TIM-3/galectin-9 could also be associated with suppression of MS.

Several effector and regulatory mechanisms that play a pathogenic role in CD4+ T cell-mediated immunopathology may be detrimental in hosts simply, because they are induced in a wrong place at a wrong time point (Table 5). Under physiological conditions (conventional infections), maintenance of CD4+ T cell-specific responses without T cell exhaustion should be vital for high levels of antibody production and CTL responses. However, such CD4+ T cell responses can play a detrimental role when the responses are prolonged or induced in pathological conditions, such as CNS autoimmunity or persistent CNS infection, where the virus can evade immune responses by infecting MHC negative cells in this immune privileged site—in the CNS, a lack of a regular lymphatic system and the presence of the BBB block conventional cellular and antibody immune responses, respectively.

Similarly, while "epitope (or determinant) spreading" (i.e., a phenomenon in which a T cell response is directed initially against one epitope, but later directed at multiple epitopes) has been proposed to enhance immunopathology in several immune-mediated diseases, including MS and rheumatic heart disease (Table 5) (Guilherme, Kalil, & Cunningham, 2006; Lehmann, Forsthuber, Miller, & Sercarz, 1992), epitope spreading may lead to recovery/protection from other immune-mediated diseases

TABLE 5 Roles of T cell exhaustion and epitope spreading

Mechanism	Physiological role	Pathological role
T cell exhaustion	Suppress immunopathology in acute infection and autoimmunity	Exacerbate chronic viral infection and cancer
Epitope spreading	Protect from pathogens and malignancies	Enhance tissue damage in autoimmunity

(Powell & Black, 2001). Furthermore, physiological (beneficial) roles of epitope spreading has been proposed in microbial infections and malignancies, where the spreading of epitopes occurs in the epitopes of pathogens or tumor antigens, contributing to more efficient clearance of microbes and tumors (Table 5) (Butterfield et al., 2003; Powell & Black, 2001).

CONCLUSIONS

The interaction among CD4+ T cells appears to be crucial in the pathophysiology of MS and its animal models. During the course of RRMS, Th1 and Th17 cells produce pro-inflammatory cytokines and play an effector role in immune-mediated tissue damage (immunopathology), whereas Th2 cells and Tregs produce anti-inflammatory cytokines and suppress the Th1/Th17-mediated immunopathology. On the other hand, Th2 immune responses may also play a pathogenic role in the development of SPMS by enhancing autoantibody production. Tregs can be a double-edged sword depending on the stage of viral infection; Tregs can play a beneficial role in virus-mediated demyelinating diseases by preventing immunopathology, while Tregs can enhance viral pathology by suppressing antiviral immune responses. CD4+ T cell subsets can be ideally controlled by "split T cell exhaustion" in which immunopathology is prevented without suppression of antiviral T cell responses. Here, personalized modulation of CD4+ T cell subsets may be a therapeutic strategy for individual MS patients whose etiology (autoimmune versus viral), clinical course (RR versus progressive), and disease stage (early, late, relapse, remission, etc.) may differ.

Acknowledgments

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