ELSEVIER

Clinical Neurology and Neurosurgery 108 (2006) 241–244

Clinical Neurology and Neurosurgery

www.elsevier.com/locate/clineuro

Neuroinflammation in multiple sclerosis: Evidence for autoimmune dysregulation, not simple autoimmune reaction

Nikolaos Grigoriadis [a,*], Savas Grigoriadis [a,b], Eleni Polyzoidou [a], Ioannis Milonas [a], Dimitrios Karussis [c]

[a] *Department of Neurology, Laboratory of Experimental Neurology and Neuroimmunology, AHEPA University Hospital, Aristotle University of Thessaloniki, 1 Stilp Kyriakidi str 54636, Thessaloniki, Greece*

[b] *Department of Neurosurgergy, AHEPA University Hospital, Aristotle University of Thessaloniki, 1 Stilp Kyriakidi str., 54636, Thessaloniki, Greece*

[c] *Department of Neurology and Agnes Ginges Center for Neurogenetics, Laboratory of Neuroimmunology, Hadassah University Hospital, Jerusalem, Ein-Karem, IL-91120, Israel*

Abstract

Both inflammatory and neurodegenerative components may contribute to the clinical profile of multiple sclerosis (MS) leading to irreversible deficits when they exceed the threshold of compensation. The mechanisms leading to tissue injury in MS are complex. Inflammation appears to be caused by overactive pro-inflammatory T-helper 1 cells, initiating an inflammatory cascade with several cellular and molecular immune components participating in the pathogenetic mechanism. Current treatments are most effective in the inflammatory phase of the disease since they may interfere with various stages of the immune cascade. Recent evidence has emerged that inflammation may not only be destructive, but may also play a part in tissue repair. This has opened up a new aspect of our knowledge of the role of the inflammatory process in MS. Data regarding the role of regulatory cells in particular, imply that specific immunomodulatory strategies that support the function of these particular cellular subpopulations may participate in the downregulation of autoimmune responses in MS.

Keywords: Multiple sclerosis; Inflammation; Regulatory cells

1. Introduction

MS is a demyelinating autoimmune disease characterized by inflammation in the central nervous system (CNS) leading to damage of the myelin sheath. The myelin sheath consists of complexes containing lipids and proteins such as myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG). Autoantibodies and autoreactive T cells against these myelin antigens have been detected in MS patients [1]. The same myelin antigens have also been used to induce experimental allergic encephalomyelitis (EAE); studies of EAE, the animal model of MS, indicate that cytokines, chemokines and adhesion molecules lead to the recruitment of leukocytes from the periphery to the CNS via a disrupted blood–brain barrier, thereby creating the appropriate inflammatory environment. The inflammatory process will eventually result in myelin as well as axonal damage and consequently in variable loss of functions [2,3]. Currently utilized immunomodulating agents in the treatment of MS, although only partially effective in terms of the clinical evolution of the disease, may exert some control over the underlying immunopathology [4].

2. Immunopathogenesis

Despite our increasing knowledge of the details of the immunological cascade of MS immunopathogenesis, it remains very complex [5,6]. A very basic concept is that MS is a $CD4^+$ T-helper 1 (Th1)-mediated autoimmune disease [7]. CD4 T cells may differentiate into Th1 and Th2 cells characterized by the production of different cytokines. Th1 cells produce pro-inflammatory cytokines such as IFNγ, TNFa,

* Corresponding author. Tel.: +30 2310 994 665; fax: +30 2310 994 689.
E-mail address: grigoria@med.auth.gr (N. Grigoriadis).

0303-8467/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.clineuro.2005.11.006

interleukin-2 (IL-2) and low levels of interleukin-10 (IL-10), whereas Th2 cells produce anti-inflammatory cytokines such as interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-13 (IL-13) and high levels of interleukin-10 (IL-10) [8].

3. The role of regulatory cells

To simply consider Th1 versus Th2 as the basis for the immunopathogenesis of MS is an oversimplification of the concept of induction versus protection. The balance between Th1 or Th2 activity has been shown to be important for the control and resolution of infectious disorders, but it is also involved in the autoimmune process [9]. Although EAE can be passively transferred by injecting Th1 cells from an EAE-induced animal into a naive host, the same cannot be done by transferring Th2 cells, a finding that gives strong support for Th1 dominance. However, when EAE goes into spontaneous remission, the Th2 cytokine IL-4 is apparently not required [10]. In addition, there are findings indicating that MBP-specific Th2 cells have the potential to induce EAE in immunodeficient mice, and that disease induction by previously activated Th1 cells cannot be prevented either by normal lymphocytes, or by previously activated Th2 cells [11].

A number of reviews dispute the view that MS is a purely Th1-mediated disease [12]. Data from MS patients, especially when considered along with the EAE results [11], does not support an absolute Th1 dominance in the disease process; on the other hand, despite the fact that the symptoms of MS can be reproduced in classical EAE in rodents, both the pure Th1 origin of the pathology in this animal model and the limited similarity between MS and EAE, raise doubts about their identity. In addition, it has been pointed out that the Th1/Th2 ratio is not markedly shifted in the T cells infiltrating sites of demyelination [13]. Interestingly enough, no differences were found in the cytokine pattern of MBP-reactive T cells between patients with MS and healthy individuals [14]. Furthermore, both elevated IFN-gamma (Th1) and IL-4 (Th2) have been reported in the serum of MS patients in the acute stage, a finding that implies the simultaneous activation of both Th1 and Th2 subpopulations [15]. Both Th2-related responses and autoantibody production have been described in EAE as well as in MS [12,16–20].

These controversial observations would suggest that the Th1 versus Th2 concept in MS is an oversimplified one, and must be interpreted with caution in discussing the pathogenesis of MS or the therapeutic value of the Th1–Th2 in vivo [21].

Despite the evidence that Th1 and Th2 are antagonistic in their role in the inflammatory cascade, other immune cell types can also block either or both Th1 or Th2 activity. These are the regulatory T cells (Tr cells); several subsets of these cells have been identified and appear to be distinct in function and phenotype (surface characteristics) from the Th1 and Th2 populations [22]. In addition, although Th1 and Th2 cells can clearly function as regulatory cells by their production of IL-10 and their ability to reciprocally regulate each other [23], they may also mediate effector functions. Such effector functions have not yet been attributed to Tr cells.

4. The $CD4^+CD25^+$ regulatory T cells

Autoimmune diseases such as MS may result from the failure of tolerance mechanisms that prevent the expansion of pathogenic T cells. This assumption arises from the observation that activated T cells may be detected in healthy individuals. Tr cells play a major role in these tolerance mechanisms; the importance of their role is exemplified by the demonstration that administration of oral antigens can induce specific Tr cells that counteract EAE. Two major Tr populations have been described so far: naturally occurring Tr (CD4+CD25+) and interleukin (IL)-10 secreting Tr cells [24]. $CD4^+CD25^+$ amount to 5–10% of the adult peripheral CD4 T cell population; they most probably arise in the thymus and resemble anergic cells in vitro. After T cell receptor-mediated stimulation, $CD4^+CD25^+$ T cells suppress the activation and proliferation of $CD4^+CD25^-$ T cells in an antigen-nonspecific manner. Their inhibitory capacity is mediated by direct cell-to-cell contact, not by cytokines [25,26]. Neonatal thymectomy or immunosuppression in mice, before these cells are generated in significant numbers, results in widespread autoimmune pathology [27]. Thus, healthy mice normally maintain a balance between naive effector cells and regulatory subsets, including $CD25^+$ and additional less well-characterized subsets of regulatory T cells.

$CD4^+$ $CD25^+$ T cells are among the best-characterized immunoregulatory subsets shown to prevent activation and effector function of activated responder T cells [27]. Increasing evidence suggests that defects in different populations of Tr cells contribute to the induction of autoimmune diseases in animal models. In a mouse model of MS, MOG 35–55-specific EAE, $CD4^+$ $CD25^+$ T cells have been shown to be able to inhibit both the onset and the progression of autoimmune demyelination induced by either active MOG35–55 immunization or adoptive transfer of autoreactive T cells [28]. These data provide further evidence that $CD4^+$ $CD25^+$ Tr cells can migrate into the CNS to mediate immune responses. Indeed, in a pilot study we have noted the accumulation of these cells in the CNS during the acute phase of PLP-induced EAE and correlated their number with a better remission (unpublished observations) (Fig. 1).

A similar defect in regulatory $CD4^+$ $CD25^{HI}$ T cells has been reported to occur in MS patients, more specifically a significant reduction in the effector functions of this regulatory T cell population. These data are among the first clear demonstrations of the functional defect of $CD4^+$ $CD25^{HI}$ Tr cells in a human autoimmune disease [29]. Coincidentally, it was reported that the levels of circulating $CD4^+$ $CD25^+$ Tr cells and $CD4^+$ $CD25^{HI}$ Tr cells were not altered in MS, and were unaffected by modulatory drugs [30]; how-

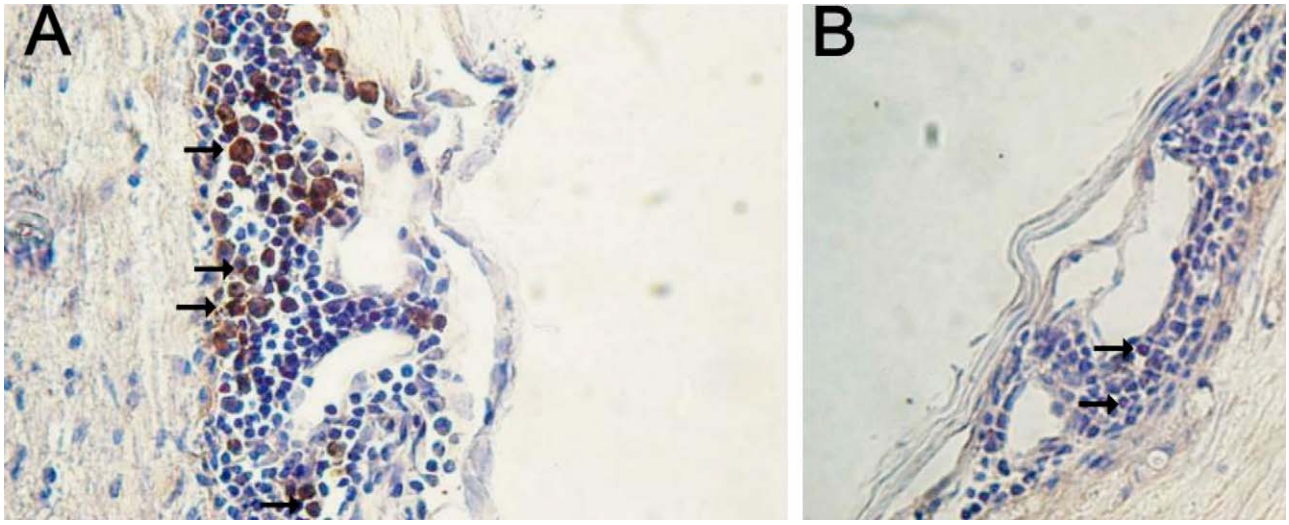

Fig. 1. CD25 (IL2R-a) immunohistochemistry: numerous positively stained infiltrating cells (arrows) in animals with a more vigorous (A) response compared to those with poor recovery (B) following EAE induction with PLP.

ever, in patients with clinically isolated syndromes (CIS) suggestive of MS, the percentage of $CD4^+$ $CD25^+$ Tr cells in CSF correlated negatively with the CSF concentration of MBP and the presence of IgG oligoclonal bands [31]. These results suggest that in the intrathecal space, the number of $CD4^+$ $CD25^+$ Tr cells may correlate with disease activity and the risk of subsequently developing MS in CIS patients. In addition, the number of gadolinium-enhanced lesions on MRI was significantly correlated with CSF cell counts, as well as the number of CD4(+)CD29(+) helper inducer and IL-2 receptor, CD25-positive activated helper T cells [32]. Another study reported that there was a correlation between MRI parameters, chemokine receptor expression and the status of circulating Tr cells in MS, but further studies are needed to discriminate between pathogenetically relevant and bystander phenomena [33].

5. A key factor in the function of Tr cells: the transcriptional regulator Foxp3

The discovery of the importance of both the transcriptional regulator Foxp3 in mouse CD4 CD25 T regulatory cell function [34,35] and of the previous observations that patients with IPEX (immune dysregulation, polyendocrinopathy, enteropathy and X-linked inheritance), a severe inflammatory disease similar to that seen in mice deficient in $CD4^+$ $CD25^+$ Tr cells, have mutations in Foxp3 [36], provided a direct correlation between an autoimmune animal model, mouse Tr cells and a human autoimmune disease. It has recently been shown that glatiramer acetate induces conversion of peripheral $CD4^+$ $CD25^-$ to $CD4^+$ $CD25^+$ Tr cells through the activation of transcription factor Foxp3. Glatiramer acetate treatment led to a significant increase in Foxp3 expression in $CD4^+$ T cells in MS patients whose Foxp3 expression was reduced at baseline. $CD4^+$ $CD25^+$ T cell lines generated by glatiramer acetate expressed high levels of Foxp3 that correlated with an increased regulatory potential [37]. Abnormalities in Foxp3 message and protein expression levels in peripheral $CD4^+$ $CD25^+$ Tr cells that are quantitatively related to a reduction in functional suppression induced during suboptimal T cell receptor (TCR) ligation, have also been reported [38].

Yet to be defined is the relationship between the two types of regulatory T cells: naturally occurring $CD4^+CD25^+$ Tr cells, which, depending on the system, may or may not require the suppressive action of IL-10, and IL-10 producing Tr cells. After transfer into lymphopenic hosts, Foxp3-transduced $CD4^+CD25^+$ T cells expressed enhanced amounts of IL-10 mRNA, comparable to those of naturally occurring $CD4^+CD25^+$ Tr cells and higher than the amounts found in untransduced cells [39]. Based on these findings it was suggested that Foxp3 directly upregulates IL-10 production, but it is likely that the cells require additional signals in vivo (besides the expression of Foxp3) for induction of the IL-10 gene. These studies did not support a direct relationship between Foxp3 and IL-10 expression [24]. Glatiramer acetate (GA) is said to induce elevated IL-10 production by Tr cells that is uniform and independent of ongoing MS treatment with IFN-beta or GA or IFN-beta + GA [39]. This finding combined with the reported Foxp3 induction in $CD4^+CD25^+$ Tr cells following GA treatment in MS patients [37] may indirectly indicate a relationship between Foxp3 and IL-10 expression, although coincidence may not be excluded.

Indications for mutations of Foxp3 in MS patients, or of any relationship between Foxp3 levels and disease activity, have not been investigated; what seems to be clear is that diminished Foxp3 levels indicate impaired immunoregulation by Tr cells that may contribute to MS. Future studies will

evaluate the effects of current treatments of MS or of therapies known to influence Tr cell function and Foxp3 expression, including TCR peptide vaccination and supplemental estrogens [38].

In the last few years there has been a rise in interest in processes that downregulate the immune response, including inhibitory receptors on cells of the immune system and regulatory T cells [40]. As more is known about distinct Tr subpopulations, it may become possible to determine their contribution to the prevention or suppression of autoimmunity and MS.

Acknowledgment

This work is part of a project supported by a Serono Hellas research grant.

References

[1] Garren H, Steinman L, Lock C. The specificity of the antibody response in multiple sclerosis. Ann Neurol 1998;43:4–6.
[2] Steinman L. Multiple sclerosis: a two-stage disease. Nat Immunol 2001;2:762–4.
[3] Grigoriadis N, Ben-Hur T, Karussis D, et al. Axonal damage in multiple sclerosis: a complex issue in a complex disease. Clin Neurol Neurosurg 2004;106:211–7.
[4] Grigoriadis N. Interferon-ß treatment in relapsing-/remitting multiple sclerosis. A review. Clin Neurol Neurosurg 2002;104:251–8.
[5] Martin R, McFarland H, McFarlin D. Immunological aspects of demyelinating diseases. Ann Rev Immunol 1992;10:153–87.
[6] Sospedra M, Martin R. Immunology of multiple sclerosis. Ann Rev Immunol 2005;23:683–747.
[7] Hafler D. Multiple sclerosis. J Clin Invest 2004;113:788–94.
[8] Abbas A, Murphy K, Sher A. Functional diversity of helper lymphocytes. Nature 1996;383:787–93.
[9] Romagnani S. Lymphokine production by human T cells in disease states. Ann Rev Immunol 1994;12:227–57.
[10] Singh V, Mehrotra S, Agarwal S. The paradigm of Th1 and Th2 cytokines: its relevance to autoimmunity and allergy. Immunol Res 1999;20:147–61.
[11] Lafaille J, Van de Keere F, Hsu A, et al. Myelin basic protein–specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. J Exp Med 1997;186:307–12.
[12] Laman J, Thompson E, Kappos L. Balancing the TH1/TH2 concept in multiple sclerosis. Immunol Today 1998;19:489–90.
[13] Cannella B, Raine C. The adhesion molecule and cytokine profile of multiple sclerosis lesions. Ann Neurol 1995;37:423–4.
[14] Windhagen A, Anderson D, Carrizosa A, et al. Cytokine secretion of myelin basic protein reactive T cells in patients with multiple sclerosis. J Neuroimmunol 1998;91:1–9.
[15] Hohnoki K, Inoue A, Koh C, et al. Elevated serum levels of IFN-gamma, IL-4 and TNFalpha/unelevated serum levels of IL-10 in patients with demyelinating diseases during the acute stage. J Neuroimmunol 1998;87:27–32.
[16] Baranzini S, Elfstrom C, Chang S, et al. Transcriptional analysis of multiple sclerosis brain lesions reveals a complex pattern of cytokine expression. J Immunol 2000;165:6576–82.
[17] Franciotta D, Zardini E, Bergamaschi R, et al. Interferon-gamma and interleukin-4 producing T cells in peripheral blood of multiple sclerosis patients. Eur Cytokine Netw 2000;11:677–81.
[18] Genain C, Cannella B, Hauser S, et al. Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nat Med 1999;5:170–5.
[19] Nakashima I, Fujihara K, Misu T, et al. Significant correlation between IL-10 levels and IgG indices in the cerebrospinal fluid of patients with multiple sclerosis. J Neuroimmunol 2000;111:64–7.
[20] Spadaro M, Amendolea M, Mazzucconi M, et al. Autoimmunity in multiple sclerosis: study of a wide spectrum of autoantibodies. Multiple Sclerosis 1999;5:121–5.
[21] Sinigaglia F, D'Ambrosio D, Rogge L. Type I interferons and the Th1/Th2 paradigm. Dev Comp Immunol 1999;23:657–63.
[22] McGuirk P, Mills K. Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. Trends Immunol 2002;23:450–5.
[23] Sher A, Coffman R. Regulation of immunity to parasites by T cells and T cell-derived cytokines. Ann Rev Immunol 1992;10:385–409.
[24] O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. Nat Med 2004;10:801–5.
[25] Jonuleit H, Schmitt E, Stassen M, et al. Identification and functional characterization of human $CD4^+CD25^+$ cells with regulatory properties isolated from peripheral blood. J Exp Med 2001;193:1285–94.
[26] Baecher-Allan C, Brown J, Freeman G, et al. $CD4^+$ $CD25^+$ high regulatory cells in human peripheral blood. J Immunol 2001;167:1245–53.
[27] Shevach E. Regulatory T cells in autoimmmunity. Ann Rev Immunol 2000;18:423–49.
[28] Kohn A, Miller S. Role of ICAM-1 and P-selectin expression in the development and effector function of $CD4^+$ $CD25^+$ regulatory T cells. J Autoimmun 2003;21:261–71.
[29] Viglietta V, Baecher-Allan C, Weiner H, et al. Loss of functional suppression by $CD4^+$ $CD25^+$ ʳegulatory T cells in patients with multiple sclerosis. J Exp Med 2004;199:971–9.
[30] Putheti P, Pettersson A, Soderstrom M, et al. Circulating $CD4^+$ $CD25^+$ T regulatory cells are not altered in multiple sclerosis and unaffected by disease-modulating drugs. J Clin Immunol 2004;24:155–61.
[31] Jensen J, Langkilde A, Fenst C, et al. CD4 T cell activation and disease activity at onset of multiple sclerosis. J Neuroimmunol 2004;149:202–9.
[32] Wang H, Matsui M, Saida T. Immunological disturbances in the central nervous system linked to MRI findings in multiple sclerosis. J Neuroimmunol 2002;125:149–54.
[33] Putheti P, Morris M, Stawiarz L, et al. Multiple sclerosis: a study of chemokine receptors and regulatory T cells in relation to MRI variables. Eur J Neurol 2003;10:529–35.
[34] Fontenot J, Gavin M, Rudensky A. Foxp3 programs the development and function of $CD4^+$ $CD25^+$ regulatory T cells. Nat Immunol 2003;4:330–6.
[35] Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003;299:1057–61.
[36] Gambineri E, Torgerson T, Ochs H. T-immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. Curr Opin Rheumatol 2003;15:430–5.
[37] Hong J, Li N, Zhang X, et al. Induction of $CD4^+$ $CD25^+$ regulatory T cells by copolymer-I through activation of transcription factor Foxp3. Proc Natl Acad Sci USA 2005;102:6449–54.
[38] Huan J, Culbertson N, Spencer L, et al. Decreased FOXP3 levels in multiple sclerosis patients. J Neurosci Res 2005;81:45–52.
[39] Putheti P, Soderstrom M, Link H, et al. Effect of glatiramer acetate (Copaxone®) on $CD4^+$ $CD25^+$ high T regulatory cells and their IL-10 production in multiple sclerosis. J Neuroimmunol 2003;144:125–31.
[40] Jiang H, Chess L. An integrated view of suppressor T cell subsets in immunoregulation. J Clin Invest 2004;114:1198–208.