The Essential Role of T cells in Multiple Sclerosis: A Reappraisal

Cris S. Constantinescu, Bruno Gran

Multiple sclerosis is an inflammatory demyelinating disease of the central nervous system in which destruction of myelin and nerve axons has been shown to be mediated by immune mechanisms. Although the focus of research has been traditionally on T cells as key mediators of the immunopathology, more recent efforts at understanding this complex disorder have been directed increasingly at other cellular and humoral elements of the immune response. This review is a reappraisal of the crucial role of T cells, in particular the CD4+ helper T-cell subset, in multiple sclerosis. Recent evidence is discussed underlining the predominant contribution of T-cell–associated genes to the genome-wide association study results of multiple sclerosis susceptibility, the loss of T-cell quiescence in the conversion from clinically isolated syndrome to clinically definite multiple sclerosis, and the fact that T cells represent the main target of effective immunomodulatory and immunosuppressive treatments in multiple sclerosis. *(Biomed J 2014;37:34-40)*

Dr. Cris S. Constantinescu

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Multiple sclerosis (MS) is a chronic immune-mediated inflammatory and neurodegenerative disease of the central nervous system (CNS) which affects predominantly young adults and represents a leading cause of neurological disability in this age group.[1]

The clinical course of MS can have several forms. The most common form at presentation is the relapsing remitting (RR) MS, manifesting as recurrent attacks (relapses) of neurological dysfunction followed by periods of remission. After a variable period of time, this is followed, in about 50% of patients, by a gradual progression with or without superimposed relapses called secondary progressive (SP) MS. A minority (about 15%) of patients have a progressive form from the onset, called primary progressive (PP) MS, and a very small number have relapses during this continuous progression, representing a form called progressive relapsing (PR) MS.[1]

MS can involve any part of the CNS and the common manifestations are sensory, motor, visual disturbances, bladder and bowel disturbance, and balance problems.[1] Neuropathic-type pain and cognitive disturbances are also quite common and increasingly recognized. An MS-specific and difficult-to-explain fatigue is present in a large number of patients.[2]

MS pathogenesis

In terms of pathogenesis, MS is a very complex disease.[1,2] Every cell type of the immune system, serving the cellular and humoral, the innate and adaptive immune responses, is involved in the orchestration of the inflammatory demyelinating damage. Likewise, although the oligodendrocyte and myelin sheath are considered the main target of the pathological process, any cellular element of the CNS can be affected by MS. Moreover, in the experimental autoimmune encephalomyelitis (EAE),[3,4] an imperfect but helpful model of MS,[5] there is evidence of processes outside of the CNS that are involved in the pathogenesis. For example, inflammatory cells cross the blood–brain barrier (BBB) from the peripheral circulation, and blockade of their entry can suppress inflammatory demyelination both in EAE and in MS.[6,7] Even the lung[8] and gut[9,10] have been shown to contribute to the pathogenesis of MS (or at least in the animal model, EAE).

From the Clinical Neurology Research Group, Division of Clinical Neuroscience, University of Nottingham School of Medicine, Queen's Medical Centre, Nottingham, UK
Received: Dec. 10, 2013; Accepted: Feb. 24, 2014
Correspondence to: Dr. Cris S. Constantinescu, Division of Clinical Neuroscience, University of Nottingham School of Medicine, Queen's Medical Centre, Nottingham, UK. NG7 2UH, UK. Tel: 44-115-8231443; Fax: 44-115-9709738; E-mail: Cris.constantinescu@nottingham.ac.uk
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T cells

T cells have been at the center of research in MS immunology for a long time, and various interventions targeting them have been considered. After the demonstration that transfer of T cells is sufficient to induce adoptive transfer EAE,[11] the specific characteristics of encephalitogenic T cells have been investigated in this experimental model and in MS itself. It was discovered, for example, that these cells have restricted T-cell receptor Vβ or Vα region usage,[12,13] which could become a target for therapeutic intervention. Such restricted T-cell receptors were also shown to be shared between several autoimmune diseases [e.g. EAE and experimental autoimmune neuritis (EAN)], leading to the hypothesis that some T-cell receptors have special propensity for mediating autoimmunity.[14]

Th1, Th2, Th17

In 1986, Mosmann and Coffman[15] put forward the concept of distinct T helper cell subsets, in large part reciprocally inhibitory, Th1 and Th2, the former producing interferon gamma (IFN-γ) and the latter, interleukin (IL)-4 (and associated cytokines IL-5 and IL-13). These T-cell subsets serve primarily the cell-mediated and humoral immunity, respectively. It was shown later that IL-4 is essential for the development of Th2 responses, and the heterodimeric cytokine IL-12, composed of a p40 and a p35 subunit, is essential for Th1 development.

There is a Th1/Th2 dichotomy in EAE. Encephalitogenic T cells produce IFN-γ, and myelin basic protein (MBP)-reactive T cells of MS patients produce more IFN-γ than the T cells of healthy controls.[16] We have shown that EAE-resistant mice produce Th2 responses and neutralization of IL-4 abrogates their resistance, whereas EAE-prone mice produce IFN-γ and neutralization of IL-12 prevents EAE.[17] However, the discovery of the related cytokine, IL-23, which shares p40 with IL-12 and has a unique p19 subunit, led to the re-examination of the role of IL-12 in autoimmune demyelination, and it was shown, through a series of experiments that IL-23 and not IL-12 is required for EAE induction.[18-22] Analysis of the effects of IL-23 led to the discovery of a new subset of T cells, Th17, that produce large amounts of the inflammatory cytokine IL-17.[23]

It was perhaps not surprising that *in vivo*, the T-cell biology is more complex than a simple dichotomy. In addition to the Th17 subset, different classes of regulatory T cells (Treg) are involved in MS. They prevent autoimmunity in normal circumstances,[24] but are deficient in MS and possibly other immune-mediated diseases.[25] Interestingly, Treg and Th17 have closely related developmental pathways. Transforming growth factor beta (TGF-β) is a stimulus for both subsets, but the presence or absence of inflammatory factors that include IL-6 and IL-1, and possibly other cytokines, leads to Th17 or Treg development, respectively.[26] There is plasticity in the Th17/Treg subset, with the possibility of Treg becoming inflammatory Th17 in a permissive environment, as shown with Toll-like receptor 2 (TLR2) stimulation.[27]

In MS, we and others have shown that Th17 cells are up-regulated, and that cells expressing both IL-17 and IFN-γ (Th1–17) are the most up-regulated in MS relapse.[28] This group of cells has been shown to inflict most damage to the BBB.[29]

The above data suggest that it would make sense to neutralize the p40 subunit that is shared by both IL-12 and IL-23; this would block both Th1 and Th17 development. This was attempted in MS in two phase II trials. One (ABT-874, briakinumab) was marginally positive; however, the effect was not deemed sufficient to warrant continued development of the drug as monotherapy.[30] The other study did not show a significant effect of the anti–IL-12/23p40 antibody (CNTO 1275, ustekinumab).[31] The reasons for this failure may be complex and have been discussed,[32] but there are several points to consider.

First, one needs to look at the characteristics of the trial patients. This was a phase II trial,[31] and safety was an important outcome measure. This allowed inclusion of patients with high levels of disability [an expanded disability status scale (EDSS) score up to 6.5]. Although it has been argued that their MS was too advanced and that is the reason why the antibody did not work,[30] the opposite argument can be made. The median EDSS score was 2.5 and the median disease duration less than 2 years. One can argue that since these subjects had few relapses (median number 0) and magnetic resonance imaging (MRI) events and there was no change in the EDSS scores in any of the five arms of the study, that is, placebo and various doses of anti–IL-12 antibody (median EDSS change was 0), any positive effect of the antibody could not be demonstrated, because the control group also had no disease activity. Erratic BBB penetration of the antibody is also not excluded.

Aside from these trial-specific limitations, more generalizable immunological considerations could be made. Although IL-12 has been shown to induce relapses[33] and overcome CD40–CD40 ligand interaction blockade in EAE,[34] when administered early in EAE, it is protective via induction of IFN-γ.[35] Thus, IL-12 blockade in the patients with early MS could have deprived them of the early protective effects of IFN-γ. We discuss Th1 cells and IFN-γ further below. Recent studies have shown that the T cells mediating MS can be heterogeneous, with Th17 cells predominating in some individuals and Th1 cells in others. This has implications in terms of response to therapies such as interferon beta (IFN-β). Patients with a Th17 profile (higher serum IL-17) did not respond favorably to IFN-β treatment. This mirrored the findings in EAE, where Th1- and Th17-mediated EAE were exacerbated or suppressed by IFN-β treatment, respectively.[36]

In addition to strengthening the case for stratified treatment of MS, these results underscore the possibility that inflammatory demyelination in MS may be mediated by cell types that do not belong to the conventional Th1 and Th17 phenotypes. The recent discovery of the role of T cells that produce granulocyte-macrophage colony stimulating factor (GM-CSF) in EAE[37,38] leads to the possibility of targeting this inflammatory cytokine/growth factor in MS. Indeed, a phase I clinical trial in relapsing remitting multiple sclerosis (RRMS) and secondary progressive multiple sclerosis (SPMS) with superimposed relapses is exploring the safety, feasibility, and immunological consequences of this approach (NCT 01517282).

In support of the heterogeneity of effector T cells in MS, we need to remember the data of the IFN-γ treatment trial, where 7 of 18 patients had relapses triggered by the treatment, while the others did not.[39] The trial rationale was based on a protective effect of IFN-γ in EAE. Thus, outbred humans with MS, unlike inbred mice with EAE, respond differently to IFN-γ. We confirmed this heterogeneity in genetically heterogeneous marmosets with EAE, where IFN-γ had different effects in different animals.[40] The complexity and discrepancies of the role of IFN-γ in EAE have been recently discussed.[41]

Regulatory T cells

As stated above, Treg[42] are cells that prevent or suppress autoimmunity in normal circumstances[43] and are deficient in a variety of autoimmune diseases including MS.[44,45] There are several types, but the most studied and probably the most potent type is the helper T cells expressing the transcriptional regulator foxp3, which also represents their signature marker.[44,45] There are natural Treg (nTreg), which develop in the thymus and are involved in immunological tolerance, and induced Treg (iTreg), which could be targets for therapeutic intervention. As advances are made in the techniques for expanding these cells and enhancing their suppressing potential *in vitro*, cellular therapy is being considered now, in particular, for transplantation.

Whether Treg cellular therapy will be successful is difficult to predict, but the risk of transdifferentiation to Th17 needs to be taken into account. Several factors, for example TGF-β[26] or aryl hydrocarbon receptor,[46,47] may induce either Treg or Th17 depending on the microenvironment, and inflammatory factors in this environment including TLR ligands,[27,48] IL-6,[26,48] IL-12 and IFN-γ,[49] may either favor Th17 development or lead to unresponsiveness of effector T cells to Treg suppression, a phenomenon that is more prominent in MS than in healthy controls.[46,50] Thus, at the present moment, there are some potential shortcomings of Treg cellular therapy, which may remain in the future.

It is much better to stimulate and increase the endogenous Treg activity and potential. Most of the current disease-modifying treatments achieve this in part, but induction of Treg is not their primary mechanism of action. Also glucocorticoids, agents with diverse, pleiotropic effects, have multiple mechanisms of actions in the treatment of MS relapses, one of which is to increase Treg cells and foxp3 expression.[51]

Observational studies carried out in Argentina on patients with MS who were also infected with intestinal parasites and had a much more benign course of their MS have shown that the principal mechanism of MS immunomodulation is enhancement of the patients' endogenous Treg responses. The ongoing Worms for Immune Regulation in MS (WIRMS, NCT 01470521) trial of controlled infection with *Necator americanus* (hookworm) in relapsing MS is based on this principle and will investigate Treg cells.

In addition to the T cell types discussed below, there are other types of T cells, and all have been implicated in MS. These include the cytotoxic (CD8+) T cells, natural killer (NK) T cells, γδ T cells, follicular helper T cells, as well as other subtypes of T helper cells such as Th9 and Th22 cells. Their roles are incompletely defined and they are not dealt with in this review.[52-56]

Recent evidence supporting the pivotal role of T cells in MS

Most studies suggest an important role for T cells in MS. Recently, attention has focused on other immunopathogenic mechanisms of this complex disease. Listed below are some of the more recent developments in the field of MS that remind us of the significant contribution of T cells to MS.

1. The major outcome of the most comprehensive genome-wide susceptibility screen for MS, which identified close to 100 genes, showed that virtually all of these genes are genes of the immune system.[57] Moreover, the majority of these genes are genes associated with T-cell function, followed by those involved in B-cell function. However, it should be stressed (see below) that the B-cell related genes are indicative of a role of B cells as antigen-presenting cells to T cells. Most recently, the integrated analysis of MS susceptibility genes [genome-wide association studies (GWAS) data] and DNAse hypersensitivity sites in 112 different cell types identified Th1, Th17, and CD8 cytotoxic T cells as those in which MS-associated genes are most active, followed by B cells and NK cells.[58]
2. Most approved disease-modifying treatments, as well as steroid treatments, have effects on T cells, these effects potentially being the key mechanism of action in some cases.[59] However, the recently developed drugs, natalizumab (Tysabri®) and fingolimod (Gilenya®) are worth discussing. Both drugs interfere with migration of T cells. The former, a monoclonal antibody, blocks

the penetration of the BBB by activated T cells that overexpress the integrin very late antigen-4 (VLA-4; $\alpha_4\beta_1$). This is because the antibody blocks the interaction between VLA-4 and vascular cell adhesion molecule-1 (VCAM-1). Although the integrin is also expressed on B cells, it is the T-cell migration effect that is therapeutic in MS. Fingolimod, a synthetic sphingosine-1-phosphate receptor ligand, also interferes with T-cell migration, but this is at the level of the secondary lymphoid organ (lymph node). Thus, a selected population of T cells, including those that mediate inflammatory damage in the CNS, are sequestered in secondary lymphoid organs and have no opportunity to migrate to the target organ, the brain, and the spinal cord. The effect of fingolimod is in great part directed at naïve and central memory T cells, and it is this T-cell effect that is used therapeutically in MS.[59]

3. Hematopoietic stem cell transplantation has been advocated as the most radical treatment for autoimmune diseases including MS.[60] The risks to the patients, although smaller than they were 15 years ago, are still considerable, and the procedure is reserved for patients with very severe disease. However, the mechanism of action was unclear until a seminal paper[61] showed that it led to a renewal of the T-cell repertoire (i.e. the repopulation of the immune system with new T cells), which may thus escape the developmental step that led to their becoming autoreactive T cells. This treatment abrogates inflammatory activity in the CNS for almost as long as patients are being followed up, meaning that a treatment that renews their T cells also abrogates inflammation.

   A recent study[62] shows that hematopoietic stem cell transplantation also induces foxp3+ regulatory T cells, and that it abrogates a class of invariant T cells. These are CD161+ CD8+ T cells, which are equivalent to the mucosa-associated invariant T (MAIT) cells. These cells are resistant to xenobiotics and produce proinflammatory cytokines such as IL-17, IFN-γ, and tumor necrosis factor (TNF). The ablation of these cells lasted at least up to 2 years after transplantation and correlated with the clinical response. Although they originate in the gut, CD161+ CD8+ T cells have the ability to home to the CNS and are present in the inflammatory lesions in the brain of people with MS.[62]
4. The first attack of inflammatory demyelination is called clinically isolated syndrome (CIS), as the patients do not fulfil the diagnostic criteria for clinically definite MS.[63] Many studies have attempted to determine the risk factors associated with conversion from CIS to clinically definite MS. Most of these studies were retrospective evaluation of prospectively collected data, and the analysis involved comparing converters to non-converters. In an important study, Corvol *et al.*, performed an unbiased gene expression analysis in naïve CD4+ cells from CIS patients at baseline and 1 year after clinically definite MS.[64] They identified several clusters, of which one, when clinical correlation was sought, captured 92% of patients who had converted to clinically definite MS. These genes were related to T-cell quiescence, and the critically important member was *TOB1*. Decreased *TOB1* expression was associated with conversion to MS, and also with progression of MS. This study, and another study investigating the mechanisms in more depth in EAE[65] demonstrate that loss of T-cell quiescence is associated with relapse of inflammatory demyelination, underscoring again the essential role of T cells in this pathological process.
5. In some novel approaches to MS immunotherapy, other immune regulatory cells may be the target. For example, the treatment with daclizumab, a monoclonal antibody against the high-affinity IL-2 receptor, was shown to induce a population of CD56 bright regulatory NK cells. However, the drug achieves this indirectly by its action of T cells. Daclizumab blocks IL-2 receptor on T cells, making IL-2 more available to NK cells to develop this regulatory NK cell subset.

B cells

The role of B cells in MS has always been investigated with great interest. After decades of focusing on the role of B cells as sources of antibodies, which can contribute to demyelination,[66] the recent success with B-cell depleting monoclonal antibody, rituximab, in RRMS,[67] followed by other anti-B cell monoclonal antibodies such as ocrelizumab, strongly suggests that other B-cell functions are more important in MS pathogenesis and their targeting better explains the effectiveness of these B-cell directed therapies. The time frame of benefit in these clinical trials is not consistent with an antibody-depletion effect. The most plausible explanation is the effect on B cells as antigen-presenting cells to encephalitogenic T cells. The fact that the Epstein–Barr virus (EBV), a virus highly implicated in MS, immortalizes B cells allowing them to be long-term antigen-presenting cells suggests that B-cell depleting therapies deplete a significant reservoir of EBV, preventing their T-cell interaction. Thus, although targeting B cell is a promising approach to MS treatment, the benefit of such targeting is through its effect on T cells.

A role for T cells in progressive MS

The above data largely refer to RR MS. In progressive MS (especially late stages), there is still low-grade inflam-

mation and degeneration (behind a closed BBB), but is not very different from non-MS controls.[68] T cells are sparse in lesions. There is evidence of activation of the innate immune system.[69] Therefore, perhaps the innate immune system should be targeted in progressive MS, ideally by agents that cross the BBB. Contrary to the concept that the immune system and T cells do not play a major role in progressive MS, there is very recent evidence of activation of Th17 cells and another group of T cells, the follicular helper T cells, in progressive MS. Whether this could/should be targeted therapeutically remains to be seen.[70] In addition, as we have seen, the role of B cells in MS is primarily explained through their antigen-presenting function to T cells. Treatment of progressive MS with B-cell depleting antibodies (ocrelizumab) currently in a phase III trial (NCT01194570), if proven effective, may lend further support to the concept that inflammation, in general, and T-cell–mediated damage, in particular, may be relevant to the progressive forms of MS.

Conclusion

To conclude, our knowledge of MS immunology has improved considerably.[71] However, despite its complexity and the contribution of many cell types to its pathogenesis, MS remains a T-cell mediated disease. Targeting pathogenic T cells either directly or indirectly and harnessing the T-cell mediated immunoregulatory mechanisms are valid therapeutic strategies in MS.

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