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Th17 cells in autoimmune demyelinating disease

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Abstract

Recently published studies in multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE) have demonstrated an association between the development of demyelinating plaques and the accumulation of Th17 cells in the central nervous system and periphery. However, a causal relationship has been difficult to establish. In fact, in reports published thus far, interleukin (IL)-17A deficiency or neutralization in vivo attenuates, but does not completely abrogate, EAE. There is growing evidence that clinically similar forms of autoimmune demyelinating disease can be driven by myelin-specific T cells of distinct lineages with different degrees of dependence on IL-17A production to achieve their pathological effects. While such observations cast doubts about the potential therapeutic efficacy of Th17 blocking agents in MS, the collective data suggest that IL-17A expression in peripheral blood mononuclear cells could serve as a surrogate biomarker of neuroinflammation and plaque formation and be a useful outcome measure for future clinical trials.

Keywords

Autoimmunity; Neuroimmunology; Experimental autoimmune encephalomyelitis; Multiple sclerosis; Interleukin-17; Th17 cells

Th lineages and autoimmune demyelination: a historical perspective

Shortly following the recognition that CD4+ T cells could be categorized into functional subsets based on their cytokine expression profile, multiple sclerosis (MS) and the animal model, experimental autoimmune encephalomyelitis (EAE), were portrayed as Th1-mediated disorders [1,2]. This characterization arose, to a large extent, from the observation that central nervous system (CNS) inflammatory infiltrates in both the human and experimental diseases are composed of activated, major histocompatibility complex class II^{hi} macrophages and microglia, as well as CD4+ T cells, suggestive of an interferon (IFN)γ-driven immune response [3]. Indeed, IFNγ-producing effector CD4+ T cells were found to be predominant in the CNS and blood of rodents with EAE and individuals with MS when compared to specimens from healthy subjects [4–9]. Myelin-specific, CD4+ Th1 cells transferred EAE to naive immunocompetent hosts, whereas Th2 cells of the same antigenic specificity were unable to do so [10,11]. Finally, intravenous administration of recombinant IFNγ triggered an acute worsening of neurological deficits in some subjects with MS [12].

Later, when the myeloid cell-derived cytokine, interleukin (IL)-12, was shown to be a potent inducer of Th1 cell differentiation, associations were found between MS disease activity and

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levels of that monokine in peripheral blood mononuclear cells, cerebrospinal fluid, and CNS specimens [13–17]. IL-12 is a heterodimeric molecule, composed of p40 and p35 chains, that stimulates T cells and natural killer (NK) cells to produce IFNγ. Messenger RNA encoding both subunits (p40 and p35) was detected at elevated levels in the brain and spinal cords of mice during exacerbations of EAE and at reduced levels during remissions [18]. Furthermore, recombinant IL-12 directly promoted the encephalitogenicity of myelin-reactive T cells in vitro and precipitated relapses when administered in vivo [19–22]. Conversely, neutralization or deficiency of the p40 subunit in vivo conferred complete resistance to EAE [23–25].

However, the causative relationship between antimyelin IFNγ responses and inflammatory demyelination of the CNS was challenged by the observation that mice deficient in certain molecules involved in the Th1 pathway were susceptible to EAE. Hence, mice deficient in IFNγ, IFNγ receptor, the IL-12p35 subunit, or the IL-12 receptor β2 chain all experienced a more severe form of EAE than their wild-type littermates [25–31]. To further complicate the issue, genetic studies have yielded conflicting results regarding the association of IL-12 and IL-12 receptor polymorphisms and risk of MS [32–35].

The above paradoxes appeared to be resolved by the discovery of IL-23. Similar to IL-12, IL-23 is a heterodimeric cytokine produced by myeloid cells that binds to a receptor expressed on subsets of T cells and NK cells. It is composed of the IL-12p40 chain complexed to a unique p19 chain [36]. Therefore, the resistance of IL-12p40 knockout mice and wild-type mice treated with anti-IL-12p40 antibodies to EAE could be secondary to suppression of IL-23, rather than IL-12, dependent pathways. Convincing evidence that this is indeed the case was provided by the phenotype of IL-23 p19 knockout mice, which fail to succumb to EAE following active immunization with myelin antigens [37]. Since IL-17A producing CD4+ T cells (referred to as Th17 cells) do not accumulate in the lymphoid tissues or target organs of IL-23p19 knockout mice following active immunization with self antigens, it was proposed that Th17 cells rather than Th1 cells are critical autoimmune effectors of inflammatory demyelinating disease [38–40]. This position was corroborated by the ability of recombinant IL-23 to promote encephalogenicity and induce IL-17A production by myelin-specific T cells in concert [39, 41].

IL-17 in MS

Similar to earlier observations regarding IFNγ, IL-17A has been reported to be expressed at relatively high levels in circulating leukocytes and cerebrospinal fluid mononuclear cells of patients with MS, particularly during relapses [42,43]. In addition, monocyte-derived dendritic cells from MS patients secreted greater quantities of IL-23 following stimulation with LPS than dendritic cells from healthy controls [44]. Perhaps most striking, transcripts encoding IL-17A were found to be elevated in MS plaques compared to brain tissues from control subjects using a microarray approach [45]. This latter finding is consistent with immunohistochemical studies demonstrating the presence of IL-17A-positive cells in active areas of MS lesions [46]. Furthermore, in a recent case report and autopsy study of a patient with aggressive relapsing remitting MS, transcripts encoding retinoic acid-related orphan nuclear hormone receptor C (RORC; the transcription factor associated with human Th17 differentiation) were upregulated in an acute lesion compared to normal appearing white matter [47].

The putative mechanism of action of IL-17 in EAE and MS

Despite a large body of literature that supports an association between Th17 cells and autoimmune demyelinating disease, relatively little data have been generated regarding the mechanism of action of IL-17A in that context. One of the best characterized functions of IL-17A is to induce production of neutrophil attracting ELR+ CXC chemokines, such as

CXCL1 and CXCL2, by a wide variety of cell types [48]. Astrocytes stereotypically upregulate ELR+ CXC chemokines in response to “danger” signals, including inflammatory stimuli [49]. Therefore, it comes as no surprise that CXCL1 and CXCL2 have been detected in astrocytes within EAE and MS lesions [50,51].

We have previously shown that myelin-reactive Th17 cells, as opposed to Th1 cells, are particularly adept at inducing CNS ELR+ CXC chemokines and recruiting neutrophils to the CNS following adoptive transfer into naive hosts [41]. Neutrophils have been implicated in enhancing cerebrovascular permeability in experimental models of stroke and encephalitis. In a separate study, we found that interactions between ELR+ CXC chemokines and their receptor, CXCR2, are critical for blood–brain barrier (BBB) breakdown, the development of neuroinflammation, and manifestation of clinical EAE in myelinimmunized mice [52]. CXCR2 knockout mice that are ordinarily resistant to EAE were rendered susceptible by reconstitution with wild-type neutrophils [52]. Such experiments suggest that activation of neutrophils with ELR+ CXC chemokines triggers BBB breakdown immediately prior to the onset of clinical EAE, which is requisite for the subsequent recruitment of large number of leukocytes to the perivascular white matter. Kebir and colleagues recently reported that human Th17 cells can also disrupt BBB tight junctions via direct effects of IL-17A and IL-22 on endothelial cells [53]. Consequently, Th17 cells were able to migrate across a brain microvascular endothelial monolayer more efficiently than Th1 cells or freshly isolated CD4+ lymphocytes.

In addition to effecting BBB breakdown, Th17 cells could promote EAE (and possibly MS) by activating neutrophils within the bone marrow and, consequently driving the mobilization of immature monocytes into the bloodstream. Granulocyte colony-stimulating factor (G-CSF) is elevated in the serum of mice injected with myelin-specific Th17 cells [41]. Bone marrow neutrophils, stimulated with G-CSF, secrete proteases that degrade chemokines (such as CXCL12) and adhesion molecules (such as α4β1 integrin and vascular cell adhesion molecule-1) that normally keep myeloid cells “anchored” within intramedullary niches [54]. Inflammatory monocytes with colony-forming unit potential expand in the circulation immediately prior to EAE exacerbations and accumulate in the CNS where they differentiate into myeloid dendritic cells [55]. CNS myeloid dendritic cells have potent antigen presenting capacities and have been implicated in epitope spreading and local Th1/Th17 polarization [21,56].

Yet another potential mechanism of action of IL-17A in EAE and MS is to stimulate production of proinflammatory molecules, such as IL-1 and IL-6, in the CNS in a positive feedback loop. IL-6-deficient mice and mice treated with IL-1 antagonists are protected from EAE induced by immunization with myelin antigens [57,58]. Transfer of myelin-specific Th17 cells increases serum IL-6 and IL-1 levels in wild-type mice [59]. The contention that IL-6 acts downstream of IL-17 is supported by the fact that IL-6-deficient mice show partial suppression of EAE induced by the transfer of myelin-specific Th17 cells [59]. It was recently reported that IL-9 is produced by Th17 cells and that IL-9 neutralization delays the onset of EAE, thereby expanding the repertoire of cytokines that participate in autoimmune pathogenesis [60]. Furthermore, IL-9 receptor-deficient mice develop a delayed and milder form of EAE with decreased numbers of IL-17A+ CD4 T cells and IL-6+ macrophages in the CNS by comparison to wild-type mice [60].

The importance of IL-17 production in the immunopathogenesis of autoimmune demyelinating disease: contributing factor versus epiphenomenon

Numerous studies cited earlier in this article have shown that myelin-specific Th17 cells are capable of inducing EAE and that Th17 cells preferentially accumulate in EAE and MS lesions. However, IL-17A is not, in and of itself, universally required for the clinical manifestation of EAE. In virtually every relevant study published thus far, treatment of myelinimmunized mice with anti-IL17A neutralizing antibodies attenuated the severity and/or delayed the onset of EAE but did not completely prevent it [39,41]. Mice deficient in IL-17A are fully susceptible to EAE, even when treated with neutralizing antibodies specific for IL-17F, a homologous cytokine to IL-17A with overlapping functions [61]. Conversely, overexpression of IL-17A in murine T cells by genetic engineering had no impact on the incidence, severity, or kinetics of clinical EAE.

The fact that IL-17A deficiency/neutralization only partially suppresses EAE could be explained by Th17 secretion of other proinflammatory mediators, such as IL-9, IL-22, and granulocyte macrophage colony-stimulating factor (GM-CSF) that ordinarily contribute to the pathogenic process in parallel to, or in synergy with, IL-17. An alternative, though not mutually exclusive, explanation is that myelin-specific T cells of non-Th17 lineages are able to compensate. Along those lines, we formerly reported that IL-12-polarized Th1 cells and IL-23-polarized Th17 cells derived from the same donor mice are equally efficient at inducing EAE by adoptive transfer [41]. The recipients of each cell type undergo a similar clinical course, based on day of onset, rate of progression, and peak severity. However, detailed analysis reveals that the two forms of EAE differ with regard to pathological features. Th1 infiltrates are enriched in highly activated macrophages and are confined to the subpial white matter, while Th17 infiltrates are enriched in neutrophils and extend into the deep parenchymal white matter. Th1-driven disease is characterized by relatively high expression of CXCL9 and CXCL10 in the inflamed spinal cord, while Th17-driven disease leads to upregulation of CXCL1 and CXCL2. Importantly, the two forms of EAE responded differently to specific immunomodulatory interventions. As expected, administration of anti-IL17 antibodies partially suppressed Th17-driven disease but had no impact on Th1 driven disease. Similarly, anti-GM-CSF was only effective in Th17-driven disease. By contrast, anti-TNFα treatment inhibited both forms of disease. This study provides a proof of concept that disparate immunopathological pathways could give rise to a similar clinical outcome. By analogy, some forms of multiple sclerosis are characterized by neutrophil-rich CNS infiltrates and elevated levels of IL-8 (suggestive of a Th17-biased response) in the cerebrospinal fluid and others by macrophage-rich infiltrates and high levels of CXCL10 (suggestive of a Th1-biased response) [62–64]. In mice immunized with myelin antigens, as well as patients with MS, the Th repertoire of autoreactive T cells is diverse. If the characteristics of the autoimmune cell population are plastic, it is likely that, in the absence of IL-17, alternate Th pathways will become predominant.

Neutralization of IL-23 as a therapeutic strategy in MS

As mentioned above, mice deficient in IL-23p19, by contrast to mice deficient in IL-12p35, IFNγ, or IL-17A, are completely resistant to EAE [37]. Furthermore, administration of monoclonal antibodies specific for IL-23p19, but not IL-17A or IL-17F, abrogates EAE [65]. Collectively, these data suggest that IL-23 is essential for the development of encephalitogenic T cells by a mechanism distinct from the induction of IL-17. Alternatively, IL-23 could promote autoimmune disease through parallel effects on non-T cells. Hence, IL-23 receptor has been

detected on activated microglia. Furthermore, IL-23 enhances IFNγ-induced STAT1 phosphorylation and CCL5 and CXCL-10 mRNA expression in microglia [66].

This discussion raises the possibility that IL-23-neutralizing agents might be more effective than IL-17A/F-neutralizing agents in suppressing disease activity in patients with MS. To that point, a phase 2, double-blind, placebo-controlled, randomized clinical trial of an antibody specific for IL-12p40 (that neutralizes both IL-12 and IL-23) was recently conducted in relapsing remitting MS [67]. Two hundred forty-nine subjects were randomized equally to five groups that received placebo or four different doses of anti-IL12p40 over a 19-week period. Anti-IL-12p40 treatment did not result in a significant reduction in the cumulative number of new gadolinium-enhancing T1-weighted images on serial magnetic resonance imaging scans (the primary outcome measure). Similar numbers of patients experienced objective clinical relapses in the placebo and anti-IL-12p40 groups. One explanation for the negative outcome of the trial is that the antibody did not penetrate across the blood–brain barrier and/or into the CNS parenchyma in sufficient quantities to achieve a therapeutic effect at the site of disease activity. Alternatively, stable, pathogenic Th1 and Th17 cell clones could have been established in the CNS and/or periphery prior to antibody treatment, obviating the need for polarizing factors to sustain autoimmune neuroinflammation. Finally, one must consider the possibility that the role of the IL-23/IL-17 axis in EAE does not translate into MS.

Th17-related molecules as surrogate markers of disease activity in MS

Whether IL-17A production in MS actually contributes to tissue damage or simply represents an epiphenomenon, a number of papers attest that its expression in peripheral blood mononuclear cells (PBMC) correlates with clinical and/or radiological disease activity. For example, in cross-sectional, flow cytometric analyses of patients with relapsing-remitting MS, IL-17A levels were higher in circulating leukocytes of patients with active disease (defined as individuals who have experienced a relapse within 10 days of phlebotomy) than in circulating leukocytes of healthy volunteers or individuals with inactive disease (defined as patients who have been clinically and radiological stable over 3 months prior to and 3 months following phlebotomy) [42]. Furthermore, longitudinal studies showed that patients with active disease experienced a selective reduction in the frequency of circulating Th17 cells in association with clinical recovery and stabilization following the initiation of interferon β therapy. The authors found that Th17-polarized CD4+ T cells preferentially express functional type I interferon receptors and are more vulnerable to IFN β-induced apoptosis than Th1-polarized cells [42]. IFN β could also suppress Th17 differentiation in patients with MS in an indirect manner, by suppressing IL-23 and enhancing IL-27 production by antigen-presenting cells [68–70]. Consistent with that hypothesis, mice deficient in type I interferon receptor mount enhanced Th17 responses, express reduced IL-27 levels in the CNS, and develop severe EAE following challenge with myelin antigens [71]. A reduction in the expression of Th17-related molecules in PBMC of MS patients in response to immunomodulatory treatment might not be specific for IFN β. The frequency of circulating Th17 cells and expression of transcripts encoding IL-17, RORC, and IL-23 receptor fell significantly in PBMC of MS patients following treatment with intravenous methylprednisolone for an acute relapse [72].

Conclusion

Based on the results of multiple studies, there is a clear association between expansion of Th17 cells in the periphery, their accumulation in the CNS, and the development of autoimmune demyelinating disease. IL-17A is the signature cytokine produced by Th17 cells. However, its role in the pathological process is likely to vary across different subsets of patients with MS and related disorders. Experiments in the EAE model indicate that compensatory cytokine pathways and alternative Th subsets of autoreactive T cells could compensate for the absence

of IL-17A and Th17 cells during the evolution of autoimmune demyelination. While this observation raises questions about the efficacy of IL-17 blocking agents in the treatment of MS, assays that measure levels of Th17-related molecules in PBMC are promising surrogate markers of clinical and radiological disease activity.

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