T HELPER 17 CELLS IN MULTIPLE SCLEROSIS AND EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Interleukin (IL)-17 was recently considered as an important immunological player for inflammation in the nervous system. It is produced by T helper (Th) cells that are distinct from the traditional Th1 and Th2 cell subsets and were thus designated as Th17 cells. IL-17 is a crucial effector cytokine with potent proinflammatory effects.

Although multiple sclerosis (MS) is the most common neurological disease of young adults, its pathogenesis is still incompletely understood. Animal (on experimental autoimmune encephalomyelitis EAE) and human studies have shown that T cells and inflammatory cytokines play an important role in MS pathogenesis. The presence of high numbers of IL-17-producing cells at the lesion site, their capacity to adhere to endothelium and their possibility to elicit a strong response, support a role for this subset in the pathogenesis and exacerbations of MS. Moreover, neutralization of IL-17 ameliorates clinical disease, a finding paralleled by reduced EAE severity in IL-17 deficient animals. A high IL-17 concentration in the serum of MS patients is associated with nonresponsiveness to IFN-β therapy.

Studying the molecules released by Th-17 cells in MS patients will shed more light on the MS pathology and might have major implications for potential therapeutic interventions.

Key words: multiple sclerosis, experimental autoimmune encephalomyelitis, Th-17, interleukin-17, interferon-β

INTRODUCTION

In 1986, Mosmann et al (1) proposed that CD4+ T cells differentiate into two subsets with reciprocal functions and patterns of cytokine secretion, termed T helper 1 (Th1) and T helper 2 (Th2). Later on, several investigators highlighted the existence of a neutral subset, naïve T cells (Th0), able to produce either Th1 or Th2. It is becoming increasingly clear that antigenic stimulation and the peculiar intrinsic characteristics of the milieu induce naïve T cells to proliferate and differentiate into different effector or regulatory T cells subsets with specific features such as cytokine production and functional properties (2).

Th1 cells produce large quantities of interferon-γ (IFN-γ) plus interleukin-2 (IL-2), induce delayed hypersensitivity reactions, maximize the killing efficacy of the macrophage and the proliferation of cytotoxic CD8+ T cells. Th1 cells are essential for defense against intracellular pathogens (cell-mediated immunity). IFN-γ increases the production of interleukin-12 (IL-12) by dendritic cells and macrophages and inhibits the production of interleukin-4 (IL-4), an important cytokine associated with the Th2 response. On the other hand, interleukin-23 (IL-23) is a heterodimeric cytokine which is shared with IL-12 and is an important part of the inflammatory response against infection (3).

Th2 cells produce mainly interleukin-4 (IL-4) plus interleukin-10 (IL-10), among other interleukins (IL-5, IL-9, IL-13) and stimulate B cells into proliferation, triggering the production of antibodies. IL-4 acts on helper T cells to promote the...
production of Th2 cytokines (including itself), while IL-10 inhibits IL-2 and IFN-γ in helper T cells and IL-12 in dendritic cells and macrophages.\(^4,5\)

Th2 responses are important to limit extracellular pathogens and can also counterbalance Th1 immune responses. Since Th1 cells are fully capable of secreting cytokines, they can in turn inhibit Th2 cell differentiation and vice versa. Several regulatory molecules are involved in this process, such as cytokines, cytokine receptors and transcription factors, ultimately leading to epigenetic modifications in the target T cells. In autoimmunity, a general concept held for a long time that Th1 response is associated with proinflammatory, disease-enhancing reaction, whereas a Th2 response exerts a modulatory function and can under certain circumstances protect against disease (antiinflammatory effect). However, Th2 responses also can be involved in the pathogenesis of inflammation mediated disorders, including autoimmune diseases.\(^6\)

Microbial stimuli induced expression of interleukin-17 (IL-17) together with tumor necrosis factor-α (TNF-α) in both murine and human T cells, independently of the production of Th1 and Th2 cytokines. The cumulative evidence about the existence of a distinct subset of T cells characterized by the secretion of large amounts of IL-17 has led to the proposal of a third lineage of T cells designated Th17 cells.\(^7-11\)

In autoimmune disorders and their animal models, IL-12 was considered to play a central upstream role in regulating the Th1/Th2 balance by providing the necessary signals for switching towards a Th1 response and subsequent clinical disease symptoms. Experimental studies have suggested that the IL-23/IL-17 axis might be relevant and not IL-12/Th1 axis (interleukin-23 is an important part of the inflammatory response against infection and has a unique role in promoting cellular immunity). CD4+ T cell which are expanded via IL-23 are characterized by the production of IL-17, IL-12 and IL-23 modulated T-cell differ in their characteristics in regard to autoimmune disease induction and the resulting pathology in the target organ.\(^3,5,12-17\)

DIFFERENTIATION OF TH17 CELLS

In the first stage, IFN-γ and IL-12 drive naïve T cells into Th1 pathway, whereas IL-4 initiates the differentiation of naïve T cells into Th2 cells. Further, IL-12 finalize differentiation of Th1 cells and IL-33 (an IL-1-like cytokine) finish the differentiation of Th2 cells. Various differentiation systems have confirmed that Th17 cells were a distinct lineage of secreting cytokines, cytokine receptors and transcription factors. IL-12 and IL-23 are unique role in promoting cellular immunity). CD4+ T cells differentiated via IL-23 are characterized by the secretion of large amounts of IL-17 has led to the proposal of a third lineage of T cells designated Th17 cells.\(^7-11\)

IL-12 is important for the differentiation of Th1 cells. It has two subunits, p35 and p40. Another protein, p19, which has no activity of its own, combines with the p40 subunit of IL-12 to form a unique heterodimeric cytokine called IL-23. Thus, IL-12 and IL-23 have in common the p40 subunit, but they also have unique subunits, p35 (IL-12) and p19 (IL-23).\(^18\)

The discovery of IL-23 shed light on the differentiation route of the novel T cell phenotype (Th17 cell). Although IL-23 is fundamental for survival and terminal differentiation of Th17 cells, its exact function in the induction of these cells has yet to be fully understood. This is partially due to the absence of the IL-23 receptor in the naïve T cells and its expression only in activated cells (highly purified naïve T cells cannot differentiate into Th17 cells in the presence of IL-23).\(^19\)

However, today we know that a combination of transforming growth factor-β (TGF-β) plus IL-6 induced the differentiation of naïve T cells into Th17 cells. Thus, TGF-β induces naïve T cells to develop into suppressor regulatory T cells, whereas IL-6 switches the transcriptional program initiated by TGF-β in a way that induces the development of Th17 cells. Exposure of developing Th17 cells to IL-23 not only enhances the expression of IL-17 but also induces interleukin-22 (IL-22) and suppress interleukin-10 (IL-10) and IFN-γ, which are not normally associated with the Th17 phenotype. IL-23 is essential for stabilizing Th17 cells. Th17 cells produce IL-17 in the presence of IL-6 and TGF-β. However, Th17 cells stimulated in the presence of IL-23 also promoted the expression of pro-inflammatory IL-17, but did not produce IL-10\(^20,23\).

IFN-γ and IL-4 produced by Th1 and Th2 cells, respectively, amplify the differentiation of these cells in an autocrine loop. IL-17, by contrast, is neither a growth factor nor a differentiation factor for Th17 cells; thus, it cannot amplify Th17 responses. However, a member of the IL-2 cytokine family – interleukin-21 (IL-21), which is produced in large amounts by mature Th17 cells – can, together with TGF-β, amplify Th17-cell differentiation. In absence of IL-21, the expansion of Th17 cells is defective. In short, there is also an autocrine loop for Th17 cells, but this loop TGF-β and IL-21 are major factors.\(^24-26\)

Several other cytokines may interfere with the development and proliferation of Th17 cells. The
neutralization of Th1 and Th2 cytokines, such as IFN-γ and IL-4, increases the number of IL-17-producing cells generated by stimulation with IL-23. On the other hand, it was demonstrated that IL-27, IL-25 and IL-13 have inhibitory functions. The absence of these cytokines exacerbates inflammatory processes and increases the number of Th17 cells.

Th17 cell differentiation is orchestrated by: a) stimulating cytokines (IL-6, IL-1β, TGF-β, IL-21, IL-23); b) inhibiting cytokines (IFN-γ, IL-4, IL-12, IL-10, IL-27).

An interesting aspect of human T helper cells is their great plasticity for differentiation compared with their murine counterparts. IL-17-producing T cells are promptly identified in populations of memory CD4+ T lymphocytes in humans although their commitment to the Th17 specific lineage is less clear since in humans these cells produce both IL-17 and IFN-γ. In addition, unlike the Th17 murine cells that are easily induced by the combination of TGF-β and IL-6 cytokines, this combination is completely inefficient in generating human Th17 cells. IL-21 and TGF-β are required for differentiation of human Th17 cells (30-33) (fig.1).

**FIGURE 1. Differentiation of T helper cell subsets**

List of abbreviations:
- APC – Antigen presenting cell; IFNγ – Interferon gamma; IL – Interleukin; GATA – Graphic alignment tool for comparative sequence analysis; RORγt – Related orphan receptor gamma t; STAT – Signal transducers and activators transcription; TGF β – Transforming growth factor beta, TF – Transcription factors.

**TH17 CELLS IN INFECTIONS**

The IL-17 cytokine family includes six members: IL-17A, B, C, D, IL-17E (or IL-25) and IL-17F. Th17 cells produce IL-17A, IL-17F, IL-21 and IL-22. In this subset of CD4+ T cells (Th17 cells), IL-17A is co-expressed with IL-17F suggesting that IL-17A and IL-17F may act both independently and synergistically to induce effector immune responses. IL-23 induces the expression of Th2 type cytokine and chemokines and plays a role in Th2 allergic responses (24, 25, 34-36).

IL-17A and IL-17F are key cytokines for recruitment, activation and migration of neutrophils. In addition to neutrophils, IL-17 might also dictate the migration of other important effector cell type during infection. The IL-17 is a proinflammatory cytokine that activates T cells and other immune cells to produce a variety of cytokines, chemokines and cell adhesion molecules (37, 38).

A number of pathogens induce mainly Th17 responses: a) Borrelia burgdorferi; b) Propionibacterium acnes; c) Citrobacter rodentium; d) Klebsiella pneumoniae; e) Mycobacterium tuberculosis; f) Candida albicans (7, 39-42).

**TH17 CELLS IN AUTOIMMUNE DISEASES**

Some of the Th17 cell products are found at higher levels in affected tissues in human diseases and in their respective animal models. The essential function of this T cell subset in induction and development of murine autoimmune diseases has been confirmed and its actual role in this pathology is now supported by evidence (9-12).

In studies of genetically deficient mice that specifically lacked IL-23 made the animals highly resistant to the development of autoimmunity and inflammation, whereas the loss of IL-12 did not. These results suggest that it is not IL-12 and Th1 cells that are required for the induction of autoimmune-mediated inflammation but rather IL-23. This also suggest that T cells that differentiate under the influence of IL-23 are key players in the induction of autoimmunity. This concept is supported by experiments in which the induction of inflammation and autoimmunity in mice was made possible by injecting IL-17 – producing T cells that had been induced to differentiate and proliferate in vivo by IL-23. Thus, it appears that the IL-23 – Th17 axis is a predominant pathway to the induction of autoimmune disease (6, 13, 14).

Studies on different autoimmune diseases such as allergic asthma, rheumatoid arthritis, systemic
levels in the serum of healthy controls (43-57). T cells and inflammatory cytokines play an important role in central nervous system (CNS) autoimmune diseases pathogenesis, including MS lesions. In the past a multitude of studies have addressed the role of Th1 (type 1 cytokines) and Th2 cells (type 2 cytokines) for adaptive responses in multiple sclerosis (MS) and its corresponding animal model, experimental autoimmune encephalomyelitis (EAE). In general, the notion has included that a type 1 response (with Th1 cells) represents a proinflammatory, destructive immune reaction whereas a type 2 response (with Th2 cells) reflects a modulatory, nonpathogenic immune reaction and can even protect from autoimmune disease caused by Th1-dependent mechanisms. Critical effector cytokines in a Th1 response are IFN-γ and TNF-α, which are both implicated in mediating disease pathology in MS and EAE. Similarly a Th2 response is characterized by cytokine secretion of IL-4, IL-5 and IL-13 (3-5, 62-64).

Because MS and EAE were originally considered as Th1-mediated autoimmune diseases, IL-12 secreted by antigen-presenting cells (APC) has commonly been implicated as the crucial upstream cytokine for the underlying Th1 response, the presence or absence of which determines the character of an evolving immune reaction. This view was later revised when it was realized that indeed IL-12 knockout mice (which lack the specific p35 subunit and not the p40 subunit shared by IL-12 and IL-23) are highly susceptible to EAE induction whereas IL-23 was discovered to be the upstream modulator to the pathogenic signaling pathways. Because IL-23 is crucially involved in the expansion of cells producing IL-17, this view converged with the discovery that are distinct from the traditional Th1 and Th2 cell subsets and were thus designated as Th17 cells. It is becoming clear that IL-12/Th17 axis play an important role in the pathogenesis of various human autoimmune diseases including MS (10,13-15).

Beyond the invaluable contribution to host defense, IL-17 – producing cells are thought to be essential inflammatory mediators in EAE. IL-17 rather than IFN-γ plays a crucial role in the development of EAE. It induces the expansion of other proinflammatory cytokines such as TNF-α and chemokines, attracts neutrophilic leukocytes and enhances the maturation of dendritic cells. Moreover, neutralization of IL-17 ameliorates clinical disease, a finding that is paralleled by reduced EAE severity in IL-17 deficient animals. The Th17 – chemokines pathways are essential for the development of CNS autoimmune disease. Perivascular dendritic cells found in inflammatory lesions are reported to polarize naïve CD4+ T lymphocytes into IL-17 – secreting cells under the influence of blood-brain barrier (BBB) secreted TGF-β and granulocyte - macrophage colony-stimulating factor. On the other hand, IL-17 receptor A (IL-17RA) is significantly increased in the CNS of mice with EAE compared to healthy mice, suggesting that IL-17RA signaling in glial cells can play an important role in autoimmune inflammation of the CNS and may be a potential pathway to target for therapeutic interventions (8, 59-61).

It is of interest that the Th17 cells from MS patients cross in vitro the BBB more easily than Th1 and that, in EAE, the Th17 cells infiltrate the brain parenchyma only when Th17 cells are increased compared with Th1 cells (65, 66).

Relatively few data are available on the involvement of IL-17 in the pathogenesis of human MS compared with mouse EAE, likely due to the difficulty in obtaining lesion samples. An increased frequency of mononuclear cells expressing IL-17mRNA in blood and spinal fluid of MS patients was reported. Transcriptional profiling of the genes expressed in MS lesions also demonstrated up-regulation of IL-6 and IL-17. It was reported increased levels of IL-17 and IL-8 in the spinal fluid of patients with the optico-spinal form of MS, in which neutrophil infiltration is more prominent than in conventional MS. Cytokine neutralization experiments demonstrated that IL-17 and IL-6 production from peripheral blood mononuclear cells in transverse myelitis and MS are increased. It was demonstrated a greater tendency to Th17 and Th1/Th17 response to non-specific stimulation in MS patients in relapse compared to controls and non-relapse patients. Monocyte-derived dendritic cells from MS patients secreted greater quantities of IL-23, following stimulation than...
The cells in active MS were basic protein specific healthy subjects, but was no chance in Th1 cells. Enfold in active MS compared with inactive MS or patient in Th17 cells were more abundant than CIS during their first neurological episode, Th17 cells were more abundant than CIS (78).

IL-17 had been found to be expressed in human astrocyte cultures and shown to be up-regulated by TNF-α and IL-1β. On the other hand, astrocytes can serve as a target of Th17 and IL-17. T cells, which secrete IL-17 or IL-17 and IFN-γ, infiltrate the CNS prior to the onset of clinical symptoms of EAE. Were they mediate CNS inflammation in part through microglial activation (75-77).

The frequency of Th17 cells was significantly higher in the cerebrospinal fluid of patients with relapsing remitting MS (RRMS) during relapse, in comparison to RRMS patients in remission or to patients with other non-inflammatory neurological diseases. Similarly, in patients with clinically isolated syndrome (CIS) during their first neurological episode, Th17 cells were more abundant than CIS patients with no acute symptoms. Patients with inflammatory neurological diseases other than MS also showed increased frequency of Th1 cells compared to patients with no inflammatory diseases (78).

The Th17 cell percentage increased around sevenfold in active MS compared with inactive MS or healthy subjects, but was no chance in Th1 cells. The cells in active MS were basic protein specific. Among the MS patients followed up for 12 months, the Th17 cell percentage decreased in patients whose disease status changes from active MS to inactive MS but remained stable in inactive MS patients whose disease status did not change. In contrast, Th1 cell percentage did not decrease significantly in patients whose disease status changed from active MS to inactive MS and changed randomly in inactive MS patients with stable disease. IFN-β inhibited Th17, but not Th1 cells in both MS patients and healthy subjects. Th17 compared with Th1 cells expressed more IFN-α/IL-1β during their differentiation and this expression correlated with their higher sensitivity to IFN-β (79).

It has become clear that the relationship between the Th1 and Th17 lineages is complex and they counter-regulate each other. There is also increasing evidence that cooperation and even dependency between these two responses in effecting pathologies. In view of evidence that each response has the capacity to cause autoimmune disease independently of the other and both may collaborate during an inflammatory response, efficient specific targeting of either Th1 or Th17 cells may prove potentially therapeutic for patients suffering from autoimmune disease including EAE or MS (80).

Hedegaard et al (81) have demonstrated a strong correlation between the MBP-induced CD4+ T-cell proliferation and production of IL-17, IFN-γ, IL-5 in MS patients and the production of IL-17 and IL-5 correlated with the number of active plaques on magnetic resonance images.

The data presented demonstrate that both Th1 and Th17 cells with specificity for myelin basic protein – MBP, proteolipid protein – PLP or myelin oligodendrocyte glycoprotein – MOG can induce CNS autoimmunity and that pathological heterogeneity in MS lesions might in part be due to multiple distinct myelin-reactive effector T cells.

**TH17 CELLS AND THERAPY WITH INTERFERON-B IN MS AND EAE**

Interferon-β (IFN-β) is the major treatment for RRMS. However, only about two thirds of patients with RRMS respond to treatment. Criteria to classify patients into responders and non-responders to IFN-β therapy are usually applied after 1 or 2 years follow-up using disability progression or relapses rate (82-84).

There are many purported mechanisms of action of IFN-β: a) reduces the Th1 pathologies by blocking the proinflammatory properties of IFN-γ, IL-12 and IL-23; b) inhibits the Th17 cell differentiation by reducing IL-1β, IL-23 and TGF-β (which induce Th17 differentiation) and by increasing IL-10, IL-27, IL-12 and IL-4 (which suppress Th17 differentiation); d) decreases serum matrix metalloproteinases (MMP)-8 and MMP-9 (85-91).

Axtell et al (92) examined whether the cytokine networks regulated by IFN-β influence its effectiveness as a therapy for RRMS. They induced EAE by transferring myelin-autoimmune T cells of either the Th1 or Th17 type into healthy mice and found that both Th1 and Th17 lineages mediated disease, but Th1 cells seem to produce somewhat more severe EAE. In a key experiment, treatment with recombinant human IFN-β exacerbated Th17-mediated EAE, but dampened Th1-mediated...
Pathological infiltrates of Th1 and Th17 cells were reduced in number in IFN-β-treated Th1-EAE spinal cords, but elevated in the Th17 counterparts. Effective treatment in Th1–induced EAE correlated with increased IL-10. In Th17–induced disease, the amount of IL-10 was unaltered by treatment, although, unexpectedly, IFN-β treatment still reduced IL-17 production without benefit. Next, the authors examined a group of patients with RRMS according to their relapse rates after IFN-β therapy. When the authors analyzed the cytokine profiles of serum samples taken from these subjects before IFN-β treatment, they found that serum concentrations of both IL-17F and endogenous IFN-β were elevated in those who did not respond to IFN-β compared to responders. Non-responders had worse disease with more steroid usage and more relapses than did responders. In the opinion of the authors, a high IL-17F concentration in the serum of patients with RRMS is associated with non-responsiveness to IFN-β therapy.

**CONCLUSIONS**

Every MS patient must be considered as an individual, rather than a stereotypic case of disease. To adapt therapies to personal needs, the neurologists need biomarkers that can distinguish among patients. In spite of massive efforts, the number of biomarkers reliably predicting the response of a patient with MS to a given therapy has remained scant. So far, none of the many candidate immunological, genetic and neurobiological predictive markers have made their way into routine neurological practice.

Prospective trials will determine whether the detection of cytokine such IL-17 and IFN-β will help selection of the MS patients who will be optimally responsive to IFN-β therapy.

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