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Current Views on the Roles of Th1 and Th17 Cells in Experimental Autoimmune Encephalomyelitis

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Abstract

Multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), are autoimmune demyelinating diseases of the central nervous system (CNS). Interferon-γ-producing Th1 and interleukin-17-producing Th17 $CD4^+$ T helper (Th) cells mediate disease pathogenesis in EAE and likely in MS as well. However, the relative contribution of each Th subset to autoimmune processes in the CNS remains unclear. Emerging data suggest that both Th1 and Th17 cells contribute to CNS autoimmunity, albeit through different mechanisms. A better understanding of the roles that Th1 and Th17 cells play in autoimmune inflammation will be helpful in developing new therapeutic approaches. In this review, we discuss recent findings on the roles of Th1 and Th17 cells in the pathogenesis of EAE.

Keywords

EAE; autoimmunity; IL-17; IL-23; interferon gamma; Th1 cells; Th17 cells; T-bet; ROR gamma T

Introduction

Experimental autoimmune encephalomyelitis (EAE) is a $CD4^+$ T cell-mediated demyelinating disease of the central nervous system (CNS) that is frequently used as an animal model for human disease multiple sclerosis (MS; Sospedra and Martin 2005). EAE can be induced in susceptible rodents by immunization with myelin antigens or by adoptive transfer of myelin-reactive $CD4^+$ T cells. Based on their cytokine secretion and transcription factor expression, effector $CD4^+$ T cells were initially classified in Th1 and Th2 lineages (Mosmann and Coffman 1989). Interferon (IFN)-γ-secreting Th1 cells, driven by interleukin (IL)-12, promote cell-mediated immunity against intracellular pathogens and express the lineage-specific transcription factor T-bet. Th2 cells, which develop in response to IL-4, express GATA3 as a lineage-specific transcription factor and are essential to destruction of extracellular parasites and mediation of humoral immunity by secreting IL-4, IL-5, and IL-13.

It has been widely accepted that deregulated IFN-γ-producing Th1 cells are pathogenic in MS and EAE, while Th2 cells are thought to be protective (Kuchroo et al. 2002). However, this dichotomy was brought into question when mice deficient in components of the IL-12/Th1 axis provided unexpected results. Mice lacking IL-12α (IL-12p35), IL-12Rβ2, or IFN-γ were more susceptible to EAE, while IL-12p40-deficient mice were resistant to disease, putting the Th1 paradigm in doubt (Ferber et al. 1996; Gran et al. 2002; Zhang et al. 2003). The discovery

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of IL-23, which has the IL-12p40 subunit in common with IL-12, and later on the discovery of IL-17-producing $CD4^+$ T cells (Th17) have filled an important gap in our understanding of EAE pathogenesis and autoimmunity in general. Mice deficient in IL-23 are completely resistant to EAE and have a defect in the Th17 compartment (Cua et al. 2003; Langrish et al. 2004). In addition, IL-23-treated myelin-specific $CD4^+$ T cells are more encephalitogenic than cells treated with IL-12 (Langrish et al. 2005). These data demonstrated that IL-23, but not IL-12, is critical in EAE pathogenesis.

Despite the dependence of Th17 cells on IL-23, their initial development from naïve T cells requires transforming growth factor (TGF)-β and IL-6 or IL-21, and this process is enhanced by tumor necrosis factor (TNF)-α and IL-1β (Stockinger and Veldhoen 2007; described in Fig. 1). IL-23 is required in the later stage of Th17 development for their terminal differentiation into mature effector cells (McGeachy et al. 2009). Development of the Th17 lineage is directed by two transcription factors, RORγt and RORα (Ivanov et al. 2006; Yang et al. 2008). Initial commitment to the Th17 lineage is antagonized by Th1 and Th2 cytokines, and both IFN-γ and IL-4 suppress Th17 differentiation (Harrington et al. 2005; Park et al. 2005). Th17 cells express a range of pro-inflammatory mediators including IL-17A, IL-17F, IL-22, and IL-21 (Ghilardi and Ouyang 2007).

In contrast to the crucial role of IL-23 in EAE, cytokines produced by Th17 cells (i.e., IL-17A, IL-17F, and IL-22) are dispensable for EAE development (Haak et al. 2009; Kreymborg et al. 2007). The concept that Th17 cells are the main culprits in CNS inflammation is being challenged by several reports showing that both Th1 and Th17 cells are involved in EAE but, individually, they induce and result in different pathogenic processes (Kroenke et al. 2008; Stromnes et al. 2008). The regulation of CNS autoimmunity by Th1 and Th17 cells and their individual contribution in initiating inflammation have been the focus of intense investigation. This review summarizes and discusses relevant new findings in the context of EAE.

Role of chemokine receptors expressed on T cells in EAE

Entry of primed T cells into the CNS is governed by both integrin-dependent adhesion to blood vessels and chemokine-driven migration through the blood–brain barrier (BBB). In both humans and mice, chemokine receptors are differentially expressed on helper T cell subsets. Th1 cells preferentially express the chemokine receptors CCR5 and CXCR3, while Th17 cells are enriched in the $CCR6^+$ compartment (Bromley et al. 2008). Initial studies revealed that gene-targeted disruption of CCR5 did not affect severity of disease, as $CCR5^{-/-}$ mice developed EAE similar to wild-type (WT) littermates (Tran et al. 2000). In addition, $CXCR3^{-/-}$ mice exhibited increased severity of EAE compared with WT mice, without quantitative differences in CNS-infiltrating cells between $CXCR3^{-/-}$ and WT mice (Liu et al. 2006). Together these data suggest that Th1 cells expressing CXCR3, CCR5, or both receptors might not be needed for EAE development. This was supported by the finding that despite more severe disease, lymph node T cells from $CXCR3^{-/-}$ EAE mice secreted less IFN-γ than WT EAE mice when reactivated in vitro (Liu et al. 2006). Perhaps the exacerbated disease found in $CXCR3^{-/-}$ mice is due to impaired IFN-γ production and concomitantly increased Th17 response, given the inhibitory effect of IFN-γ on Th17 cell development (Harrington et al. 2005; Park et al. 2005). Whether Th17 cells are preferentially recruited over Th1 cells into the CNS of $CXCR3^{-/-}$ EAE mice was not analyzed in that study (Liu et al. 2006). However, in another report describing more severe EAE in $CXCR3^{-/-}$ mice, neither IFN-γ nor IL-17 expression in inflamed CNS was affected, preventing the conclusion that increased susceptibility to EAE of $CXCR3^{-/-}$ mice is caused by augmented Th17 cells response (Muller et al. 2007).

The correlation of migratory abilities of Th17 cells and their expression of distinct chemokine receptors has been examined in several studies. Gene microarray analysis of Th17 cells that developed in vivo or differentiated in vitro in the presence of TGF-β and IL-6 revealed high expression of CCR6 (Hirota et al. 2007; Yamazaki et al. 2008). In addition, retroviral-mediated transduction of RORγt in naïve T cells resulted in upregulation of both IL-17 and CCR6 (Hirota et al. 2007). CCL20, a ligand for CCR6, is highly expressed by Th17 cells (Hirota et al. 2007). $CCR6^+$ Th17 cells exhibit strong chemotaxis toward CCL20, suggesting a possible positive feedback loop where Th17 cells further recruit other CCR6-expressing T cells at the site of inflammation (Hirota et al. 2007). Upregulation of CCR6 and CCL20 has been described in many inflammatory conditions, including EAE (Kohler et al. 2003; Liston et al. 2009). $CCR6^{-/-}$ mice susceptibility to EAE has been analyzed by several groups, resulting in contradictory findings. In two studies, $CCR6^{-/-}$ mice developed milder EAE when compared to WT mice (Liston et al. 2009; Reboldi et al. 2009), while three other groups described more severe EAE in $CCR6^{-/-}$ mice, with or without delayed disease onset (Elhofy et al. 2009; Villares et al. 2009; Yamazaki et al. 2008). The reasons for contradictions among these studies remain unclear, but they might be due to different $CCR6^{-/-}$ mouse strains used or different experimental protocols used to induce EAE. Even if general CCR6 deficiency has a controversial effect in EAE, lack of CCR6, specifically in Th17 cells, has a crucial impact on disease development. Th17 cells were reduced in the CNS of $CCR6^{-/-}$ EAE mice compared to WT mice, although both strains had similar Th17 responses in the periphery. These findings indicate that CCR6-mediated Th17 cell infiltration into the CNS may be important in EAE development (Yamazaki et al. 2008). Using an IL-23-driven adoptive transfer EAE model, Yamazaki and colleagues found that $CCR6^{-/-}$ encephalitogenic Th17 cells, when transferred to WT recipients, failed to migrate to the CNS and did not induce EAE (Yamazaki et al. 2008). Similarly, in an elegant study from Sallusto's group, $CCR6^{-/-}$ mice were completely resistant to EAE, which was associated with reduced ability of Th1 and Th17 cells to infiltrate the CNS despite normal differentiation of both subsets in draining lymph nodes (Reboldi et al. 2009). Susceptibility of $CCR6^{-/-}$ mice to EAE was restored to WT level by transfer of $CCR6^{+/+}$ MOG-specific 2D2 T cells, while transfer of $CCR6^{-/-}$ 2D2 T cells was inefficient. Transferred $CCR6^+$ cells primed in vivo preferentially expressed IL-17, demonstrating that $CCR6^+$ Th17 cells are necessary for disease development. Interestingly, analysis of cells infiltrating the CNS of $CCR6^{-/-}$ mice transferred with WT 2D2 T cells showed that at day20 postimmunization, the majority of infiltrating T cells were from recipient origin, i.e., $CCR6^{-/-}$ (Reboldi et al. 2009). This indicates that inflammation is triggered by a CCR6-dependent infiltration of autoreactive Th17 cells, followed by a second wave of both Th1 and Th17 cells in a CCR6-independent manner (Reboldi et al. 2009). CCL20, the ligand of CCR6, is constitutively expressed in epithelial cells of the choroid plexus. Results showing that $CD45^+$ cells accumulated at this site in $CCR6^{-/-}$ mice and did not cross the choroid plexus epithelium to access the CNS demonstrate that the CCR6–CCL20 axis is important in initiating CNS inflammation (Reboldi et al. 2009). Thus, $CCR6^+$ Th17 cells enter the CNS by interacting with CCL20 at the choroid plexus epithelium, trigger inflammation, and modify BBB permeability, enabling subsequent infiltration of other lymphocytes independently of CCR6 expression (Reboldi et al. 2009). All together, these data indicate that $CCR6^+$ Th17 cells are essential in the early phase of disease (Fig. 2).

Contributing roles of Th1 and Th17 cells in the pathogenesis of EAE

Which subset of helper T cells is most critical for the pathogenesis of EAE is still a matter of debate. Mice deficient in either RORγt or T-bet are resistant to EAE induction, supporting the view that both Th17 and Th1 cells are involved in CNS autoimmunity (Bettelli et al. 2004; Ivanov et al. 2006). Studies analyzing the phenotype of T cells infiltrating the CNS during EAE showed the presence of both Th1 and Th17 cells (Korn et al. 2007; Langrish et al. 2005); however, their relative proportions varied among different mouse strains. In B6 mice,

autoreactive Th1 cells predominated in the inflamed CNS at disease peak, whereas in SJL mice, there were more Th17 cells (Korn et al. 2007; Langrish et al. 2005). Immunization of mice with distinct MOG epitopes elicited T cell responses with a different Th1 to Th17 ratio, depending on the avidity of T cells for their cognate antigen (Stromnes et al. 2008). These data suggest that the relative contribution of Th1 and Th17 responses in CNS inflammation might vary depending on the strain of mice and immunization strategy.

In attempts to clearly define which T cell helper subset is critical in EAE pathogenesis, investigators used the adoptive transfer EAE model. In order to enrich cells in encephalitogenic Th1 or Th17 cells before transfer, splenocytes and draining lymph node cells from immunized mice were cultured in vitro in the presence of either IL-12 or IL-23, respectively. Using this approach and splenocytes from immunized SJL mice, Langrish et al. described a more severe EAE when Th17 cells were transferred compared to Th1 cells (Langrish et al. 2005). However, in another report using a similar protocol in SJL mice, autoreactive Th1 and Th17 cells were shown to induce similar clinical disability (Kroenke et al. 2008). The reason for these discrepancies is unclear and might be due to differences in protocols followed for in vitro reactivation of myelin-specific T cells before adoptive transfer. Nonetheless, because these studies did not use pure Th17 cells, one cannot exclude the possibility that contaminating Th1 cells may have contributed to the pathogenicity of adoptively transferred cells. Several reports have shown the presence of Th1 cells in IL-23-driven cultures (Fitzgerald et al. 2007; McGeachy et al. 2007). In addition, plasticity of the Th17 phenotype discussed below might also have contributed to the differences observed in these studies.

Some recent studies have argued that both Th1 and Th17 cells participate in CNS autoimmunity. In experimental autoimmune uveitis, a model with important similarities to EAE, transfer of either antigen-specific Th1 or Th17 cells can induce tissue damage (Luger et al. 2008). Kroenke et al. described similar findings in EAE. Adoptive transfer of either myelin-specific Th1 or Th17 cells drove CNS inflammation, resulting in similar clinical paralysis (Kroenke et al. 2008). However, the pathology induced by Th1 and Th17 cells was distinct. Th1-mediated CNS inflammation was characterized by infiltrating macrophages, whereas neutrophils predominated when Th17 cells were used to induce disease (Kroenke et al. 2008). Similarly, Stromnes and colleagues described a comparable capacity of myelin-specific Th1 and Th17 cells to induce EAE, although with different regional localization of CNS lesions and different clinical disease (Stromnes et al. 2008). Transfer of Th17 cells induced mainly brain inflammation in recipient mice, which developed atypical EAE, while transfer of Th1 cells led to the development of classical EAE with only spinal cord inflammation (Stromnes et al. 2008). Interestingly, transfer of MOG-specific T cells containing different proportions of Th1 and Th17 cells directed the localization of CNS inflammation. At a high Th17/Th1 cell ratio of CNS-infiltrating T cells, inflammation occurred in the brain parenchyma whereas with a lower Th17/Th1 ratio inflammation was located in the spinal cord (Stromnes et al. 2008). In addition, mice transferred with encephalitogenic Th17 cells and treated with soluble IL-17RA to neutralize IL-17 activity developed classical EAE, with spinal cord infiltration and without brain inflammation, in contrast to the atypical EAE that developed in untreated mice. Collectively, these data demonstrate that both autoreactive Th1 and Th17 cells, their balance at the site of inflammation, and their cytokines and chemokines are responsible for CNS autoimmunity. The site where pathology occurs is dependent on the ratio of Th1 and Th17 cells in CNS-infiltrating cells which could account for the variety of clinical forms of disease found in MS patients. Moreover, these data are consistent with previous findings demonstrating that the port of entry of Th17 cells during EAE is the choroid plexus (Reboldi et al. 2009).

Of particular interest is that atypical EAE, with marked brain inflammation mediated by Th17 cells, resembles disease that occurs in IFN-γ knockout (KO) mice (Willenborg et al. 1996), consistent with increased differentiation of pathogenic Th17 cells in the absence of IFN-γ

(Luger et al. 2008). Additional facts suggest that Th1 cells and IFN-γ play a role in promoting spinal cord inflammation. In a report by Lees et al., regional IFN-γ responsiveness directed whether the spinal cord or brain became inflamed (Lees et al. 2008a). The authors described that the transfer of either IL-12-stimulated WT encephalitogenic Th1 cells into IFN-γR-deficient recipients or IFN-γ KO Th1 cells into WT hosts resulted in development of atypical EAE, with prominent inflammatory infiltrates in the cerebellum and brain but not in the spinal cord. Because both spinal cord and brain of WT recipient mice expressed similar levels of IFN-γR, differential effects of IFN-γ were not due to restricted responsiveness to IFN-γ in one CNS region. These findings suggest that lesion localization is controlled by CNS response to IFN-γ. Nevertheless, the mechanism by which IFN-γ regulates localization of inflammation was not defined in this study and remained unknown. In agreement with previous results (Stromnes et al. 2008), production of IL-17 was increased in brain lesions during atypical EAE. However, the cellular source of IL-17 was host cells, indicating that IL-17-producing cells contribute to atypical disease but do not govern the location of inflammatory infiltrates (Lees et al. 2008a). The same group identified host gamma delta T cells as the main producers of IL-17 during EAE induced by Th1 adoptive transfer (Lees et al. 2008b). As a consequence, it is likely that IFN-γ signaling in brain and spinal cord induces distinct chemokine environments that can attract innate IL-17-producing cells. Accordingly, distinct expression of the IFN-γ-inducible chemokines CXCL9 and CXCL10 was observed when tissues from typical and atypical EAE were analyzed (Lees et al. 2008a). Further studies are warranted to define the precise roles of IL-17 and IFN-γ signaling in CNS environment during inflammation.

Role of IL-23 in promoting effector functions of Th17 cells

Although the discovery of IL-23 resolved major contradictions in the Th1 paradigm of EAE, it remains obscure how IL-23 controls the Th17 immune response. Shortly after the “IL-23 breakthrough”, it was found that IL-23 acts on memory but not on naïve $CD4^+$ T cells (Oppmann et al. 2000), demonstrating that IL-23 does not participate in de novo Th17 differentiation, at least in mice (Harrington et al. 2005; Park et al. 2005; Veldhoen et al. 2006).

The precise mechanism underlying the Th17-promoting effect of IL-23 remains puzzling. Initial observations suggested that IL-23 promotes expansion, survival, and lineage stability of committed Th17 cells (Veldhoen et al. 2006). Mice deficient in IL-23p19 do not develop Th17 cells upon EAE immunization (Cua et al. 2003). Upon antigenic stimulation, Th17 cells secrete a small amount of IL-17 in the absence of IL-23 (Liu et al. 2005), while for sustained IL-17 production, Th17 cells require IL-23 signaling leading to STAT3 phosphorylation and its binding to the IL-17 promoter (Chen et al. 2006). Overall, the above findings indicated that IL-23 may promote expansion and survival of Th17 cells. However, further analysis revealed that IL-23 maintains the Th17 phenotype rather than stimulating proliferation of effector/memory Th17 cells (Ciric et al. 2009; Lee et al. 2009; Stritesky et al. 2008). Work from Cua's group demonstrated that IL-23 is crucial for the encephalitogenicity of Th17 cells. They identified subsets of Th17 cells based on their distinct pathogenicity in EAE. Myelin-specific Th17 cells restimulated with a combination of TGF-β + IL-6 failed to induce EAE, while cells treated with IL-23 were highly encephalitogenic (McGeachy et al. 2007). Th17 cells stimulated with TGF-β + IL-6 were present in the CNS but in reduced number when compared to IL-23-stimulated cells. This was consistent with results showing downregulation of certain chemokines produced by nonpathogenic Th17 cells and decreased numbers of CNS infiltrating leukocytes in mice that received TGF-β + IL-6-treated cells. These data imply that the lack of pathogenicity of TGF-β + IL-6-treated Th17 cells can be either due to their failure to accumulate in sufficient numbers in the CNS or to their inability to attract other immune cells and initiate inflammation. However, even when injected into the brain of naïve recipients, TGF-β + IL-6-treated Th17 cells did not induce disease, demonstrating that the nonencephalitogenic nature

of TGF-β + IL-6-treated Th17 cells is not caused by their failure to migrate into the CNS. Finally, upregulation of anti-inflammatory cytokine IL-10 was described as a defining feature of the nonpathogenic Th17 cell subset, which may explain, at least in part, why these cells, even when present in the CNS, do not induce inflammation (McGeachy et al. 2007). However, treatment of recipient mice injected with TGF-β + IL-6-treated Th17 cells with anti-IL-10 Abs did not lead to development of EAE, suggesting that other factors produced by Th17 cells that are essential for their effector functions might be affected by exposure to TGF-β + IL-6 (McGeachy et al. 2007). Taken together, these data demonstrate a crucial role for IL-23 in promoting effector functions of Th17 cells.

Like IL-23-deficient mice, IL-23R KO mice are completely resistant to EAE induction (Awasthi et al. 2009; McGeachy et al. 2009). Using an elegant model of mixed bone marrow chimera mice in which 50% of donor bone marrow was IL-23R$^{+/+}$ CD45.1^{+} and 50% was IL-23R$^{-/-}$ CD45.1^{-}, McGeachy and colleagues analyzed the development of Th17 immune response after immunization. These chimeric mice were fully susceptible to EAE. Analysis of CNS infiltrating cells revealed that IL-23R$^{-/-}$CD4^{+} T cells failed to accumulate in the CNS while WT CD4^{+} T cells infiltrated the CNS normally (McGeachy et al. 2009). Similar data were recently published by Becher's group (Gyulveszi et al. 2009). In addition, when stimulated in vitro, myelin-specific production of IL-17 by IL-23R$^{-/-}$ CD4^{+} T cells infiltrating the CNS was impaired, suggesting that IL-23 signaling is necessary during Th17 differentiation (McGeachy et al. 2009). Early differentiation of Th17 cells was not affected by IL-23R deficiency until day5 postimmunization. However, at later time points, the percentage of IL-23R-deficient Th17 cells in draining lymph nodes failed to increase and declined over time, indicating that IL-23 signaling is required during development of Th17 cells (McGeachy et al. 2009). These data suggest that the main site of action of IL-23 is in peripheral lymphoid organs, given that IL-23R-deficient Th17 cells failed to infiltrate the CNS and remained in lymph nodes. When transferred into IL-23-deficient hosts, WT T cells induced impaired antigen-specific delayed-type hypersensitivity (DTH) in comparison with the response found in WT hosts. The addition of exogenous IL-23 at the site of challenge partially restored DTH response, suggesting that IL-23 can also act on already developed Th17 cells at the site of inflammation (McGeachy et al. 2009). Moreover, these results are consistent with previous findings demonstrating that IL-23-deficient mice, which are resistant to EAE induction, develop disease when exogenous IL-23 is delivered into the CNS (Cua et al. 2003). Further analysis indicated that lack of IL-23R in Th17 cells prevented their terminal differentiation into functional effector cells. IL-23R-deficient Th17 cells were susceptible to apoptosis and maintained an immature phenotype, with failure to down-regulate IL-2 and CD27 and to upregulate IL-7Rα (McGeachy et al. 2009). In summary, these data uncover a requirement for IL-23 early in Th17 development and their terminal differentiation into effector T cells in vivo.

Plasticity of Th17 phenotype

The unexpected observations by different groups that T cells co-express IFN-γ and IL-17 in vivo under both homeostatic and inflammatory conditions have opened a debate on the stability of the Th17 phenotype (Acosta-Rodriguez et al. 2007; Ivanov et al. 2006; Korn et al. 2008; Suryani and Sutton 2007). Cells secreting both IL-17 and IFN-γ are usually detectable in the CNS of EAE mice as well (Abromson-Leeman et al. 2009; Korn et al. 2008; Suryani and Sutton 2007). It remains to be established if these cells represent a stable subset or a transitional phenotype between Th17 and Th1 cells. Interestingly, genetic profiling of cloned encephalitogenic T cells from immunized TCR transgenic mice revealed both T-bet^{+}/RORγt^{-} and T-bet^{+}/RORγt^{+} cells (Abromson-Leeman et al. 2009). When restimulated with cognate antigen, T-bet^{+}/RORγt^{-} clones produced exclusively IFN-γ, while T-bet^{+}/RORγt^{+} clones produced both IL-17 and IFN-γ. In addition, T-bet^{+}/RORγt^{+} clones responded to exogenous IL-12 or IL-23 by either downregulating or upregulating IL-17, respectively, while

their expression of T-bet and RORγt remained stable. Both T-bet^{+}/RORγt^{-} and T-bet^{+}/RORγt^{+} cells induced EAE when transferred to naïve recipients, indicating that both cell types likely contribute to CNS autoimmunity in vivo (Abromson-Leeman et al. 2009). These data suggest that the relative expression of T-bet and RORγt within the same cell and cytokines present in the milieu may cause the phenotype instability of Th17 cells. Interestingly, in the abovementioned study, all clones producing IL-17 also expressed T-bet (Abromson-Leeman et al. 2009). These results are consistent with previous reports demonstrating the important role of T-bet in Th17 cell biology (Gocke et al. 2007; Yang et al. 2009). Furthermore, like IL-17 single positive Th17 cells, IL-17 and IFN-γ double producers have been found at lower frequency in IL-23R-deficient mice, suggesting that they more likely originate from Th17 rather than Th1 cells (McGeachy et al. 2009).

The mechanism leading to the development of cells producing both IL-17 and IFN-γ is not known. However, IL-12 and IL-4 have been found to have a profound effect on committed Th17 cells by readily converting them into Th1 and Th2 cells, respectively (Lee et al. 2009; Lexberg et al. 2008). Consistent with this observation, the conversion of Th17 cells into Th1 or Th2 cells was followed by upregulation of T-bet or Gata-3, respectively (Lee et al. 2009; Lexberg et al. 2008). The generation of IL-17 reporter mice allowed investigators to analyze the phenotype stability of pure Th17 cells. Lee et al. described that repeated stimulation of Th17 cells gave rise to a heterogeneous cell population. Depending on the cytokines used during restimulation, Th17 cells either retained high expression of IL-17 or expressed variable levels of IL-17 and IFN-γ (Lee et al. 2009). The presence of TGF-β was necessary to maintain IL-17 expression and to prevent IFN-γ production by Th17 cells. In the absence of TGF-β, both IL-12 and IL-23 stimulated Th17 cells shifted toward the production of IFN-γ and progressive extinction of IL-17 expression, yet with different kinetics (Lee et al. 2009). When transferred into RAG$^{-/-}$ mice, Th17 cells were a potent inducer of colitis. Consistent with in vitro findings, a large proportion of transferred cells lost expression of IL-17 and became IFN-γ producers, demonstrating the plasticity of Th17 lineage in vivo (Lee et al. 2009). Similar data were obtained in a transfer model of type 1 diabetes. Transferred islet-specific Th17 cells caused diabetes, but the majority of them converted to IFN-γ-producing cells (Bending et al. 2009). Interestingly, diabetes induced by transferred Th17 cells was prevented when recipient mice were treated with anti-IFN-γ but not with anti-IL-17. However, IFN-γ blockade did not prevent the Th17 to Th1 conversion, suggesting that other factors, possibly IL-12, stimulated this phenotype shift.

This late instability has been attributed to epigenic modifications in differentiated T helper subsets. Methylations of DNA-associated histones regulate accessibility of elements present in the promoters that bind transcription factors. Trimethylation of lysine 4 on histone H3 (H3K4me3) is a permissive mark associated with active or poised promoters whereas trimethylation of lysine 27 on histone H3 is associated with silenced genes. Elevated permissive marks in IFN-γ, IL-4, or IL-17 genes were associated with Th1, Th2, or Th17 cells, respectively. Reciprocally, repressive marks in these cytokine genes were found in the opposing T helper subset (Wei et al. 2009). In addition to IL17, high amounts of permissive marks were selectively detected in IL-1R and IL-17R promoters in Th17 cells. Interestingly, analysis of DNA methylation in the promoter of lineage-specific transcription factors T-bet and Gata-3 in Th17 cells showed both permissive and repressive marks, demonstrating that these genes are in a poised state and can be further activated or repressed (Wei et al. 2009). These findings are in agreement with the observed profound effect of IL-12 and IL-4 on committed Th17 (Lee et al. 2009; Lexberg et al. 2008). However, because both the Rorc and IL-17 loci have repressive marks in Th1, it is unlikely that Th1 cells can convert to Th17 cells (Wei et al. 2009). Overall, these studies describe a more complex view of lineage determination in helper T cells. The observed unidirectional plasticity between Th17 and Th1 cells might be an important regulatory mechanism limiting tissue damage caused by IL-17.

Conclusions

Over the past decade, a significant advance has been made in our knowledge of CNS autoimmunity. Discovery of the IL-23/Th17 axis resolved discrepancies in the Th1 paradigm and Th17 cells have appeared as major players in immune-mediated CNS disease. However, inflammatory cytokines produced by Th17 cells such as IL-17A, IL-17F, and IL-22 were found to be dispensable for disease induction, raising issues on the exact role played by Th17 cells in EAE. With the evidence of plasticity between helper T cells subsets in mind, it is now clear that production of a single cytokine is not sufficient to define the Th17 subset. The current concept is that both IL-17 and IFN-γ play a role in CNS pathogenesis. How Th1 and Th17 cells interact in vivo among themselves and with the other regulatory and effector T cell subsets remains to be fully elucidated. The different clinical forms of multiple sclerosis are likely to be caused by diverse subsets of helper T cells, their relative proportion at the sites of inflammation, and their predominant production of either IL-17 or IFN-γ. Successful therapeutical strategies in MS might require characterization of the immune response in a given patient to design effective treatment protocols on an individual basis.

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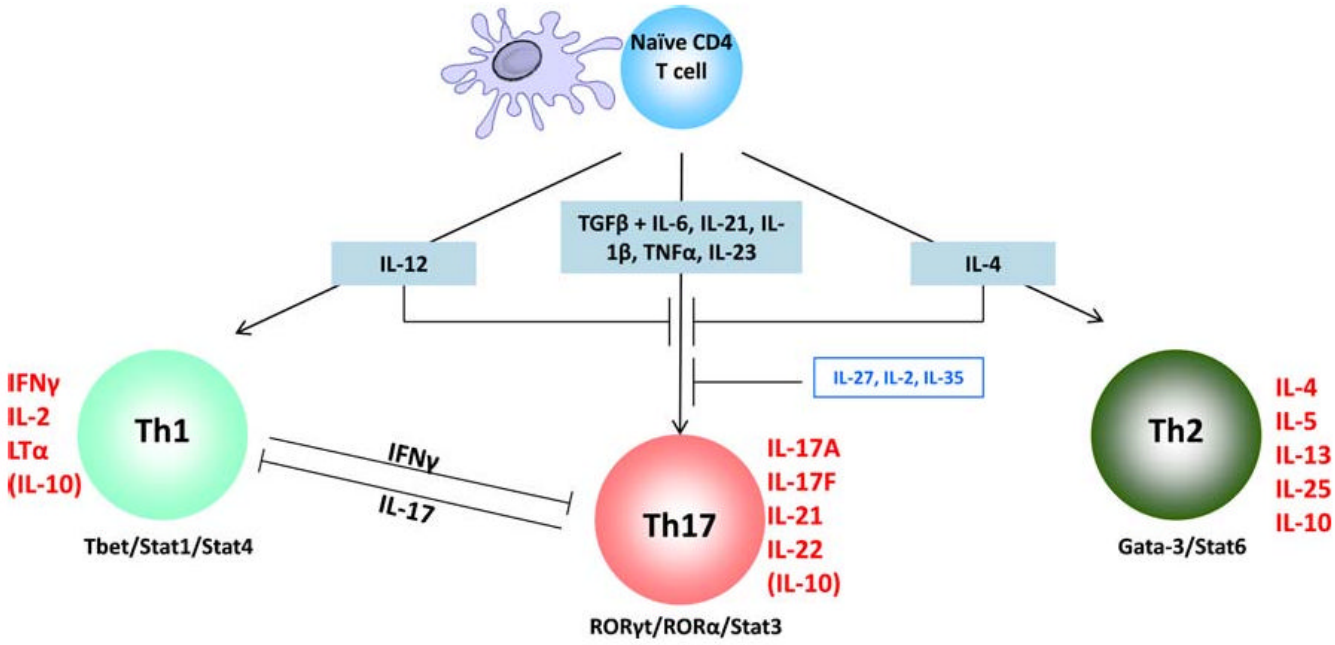

Fig. 1.

Differentiation of effector T helper subsets. After activation by professional antigen-presenting cells, naïve $CD4^+$ T cells differentiate toward Th1 cells in the presence of IL-12. Th1 cells upregulate IFN-γ via Stat4, leading to IFN-γ-mediated Stat1 activation and induction of the Th1 lineage transcription factor T-bet. Th2 cells differentiate in response to IL-4, which activates Stat6, resulting in induction of GATA3. Th17 cell subset develops in response to IL-6 or IL-21 and TGFβ. This differentiation is enhanced in the presence of IL-1β and TNFα. IL-6 activates Stat3 and the lineage-determining transcription factors RORγt and RORα. Th17 differentiation is strongly inhibited by IFN-γ, IL-4, IL-27, IL-2, and IL-35. In addition, IL-17 inhibits Th1 polarization, expression of IFN-γ, and the transcription factor T-bet (O'Connor et al. 2009)

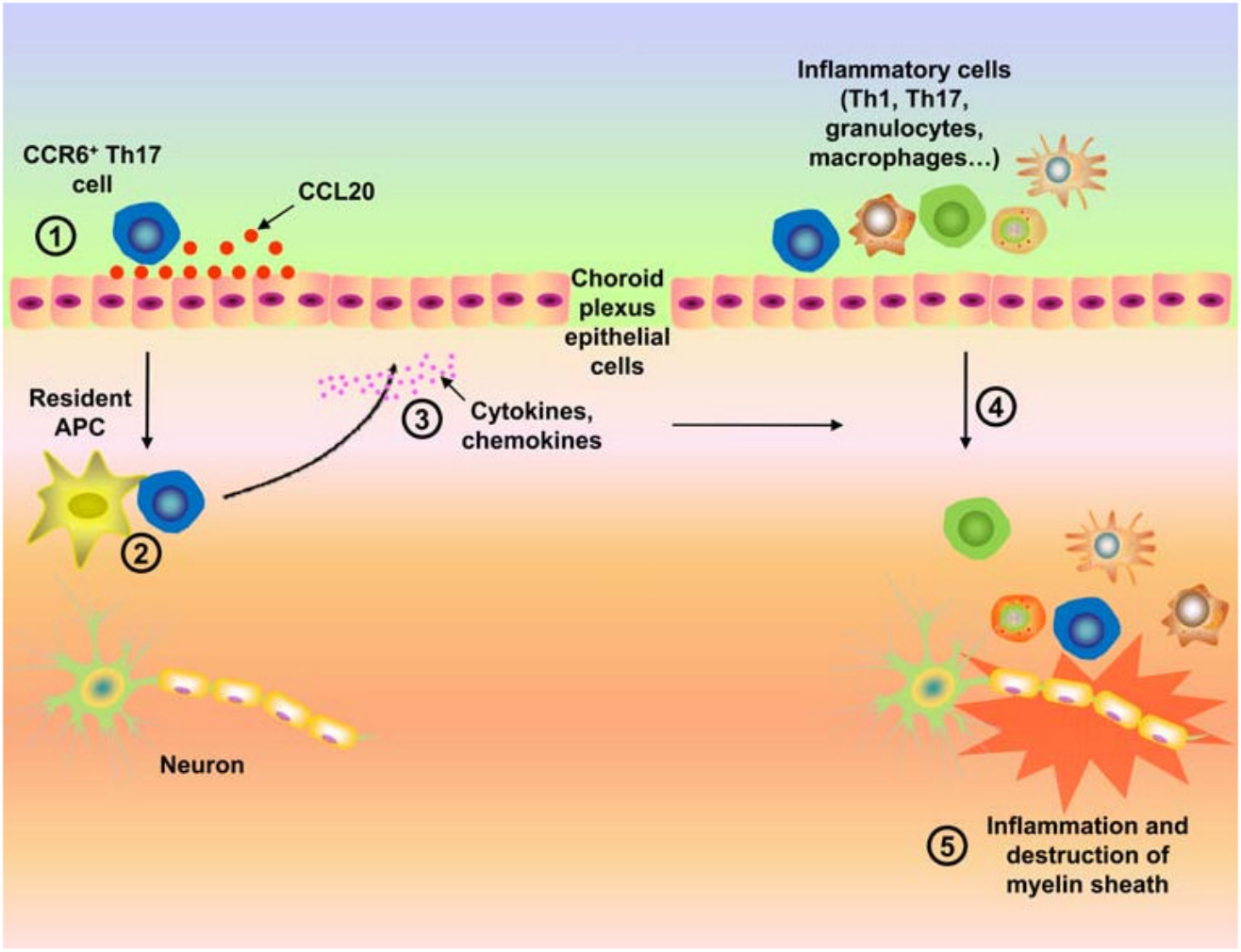

Fig. 2.

CCR6–CCL20-dependent entry of Th17 cells into the CNS. $CCR6^+$ Th17 cells interact with CCL20 expressed by the epithelium of the choroid plexus and enter the CNS. After reactivation with resident antigen-presenting cells and myelin antigens, $CCR6^+$ Th17 cells secrete several cytokines and chemokines that initiate inflammation by modifying BBB permeability and attracting other immune cells. Further infiltration of various leucocytes, including Th1 cells, Th17 cells, granulocytes, and macrophages, amplifies the inflammation process that mediates destruction of the myelin sheath