IL-17A in Autoimmune Inflammation of the Central Nervous System: A Promising Therapeutic Target in Multiple Sclerosis?

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Abstract: Discovery of the IL-23/Th17 axis brought to an end the era of the Th1/Th2 paradigm and radically advanced our understanding of autoimmunity. Experimental autoimmune encephalitis (EAE), an animal model of multiple sclerosis (MS), was highly instrumental in discovery of the IL-23/Th17 axis and the ensuing rapid progress in the field. Despite an early start and subsequent intense focus on the role of this axis in EAE and MS, some basic questions remained controversial, such as the role of IL-17A, a signature cytokine of Th17 lineage. Copious production of IL-17A combined with its pro-inflammatory effects offered a plausible explanation for the pronounced encephalitogenicity of Th17 cells. A body of experimental data from a number of laboratories demonstrated its important, if not essential, role in EAE, thus pointing to this cytokine as a promising therapeutic target in MS. Findings that MS lesions contain high percentages of Th17 and Tc17 cells and large quantities of IL-17A, similar to EAE, further supported this view. Unexpectedly, two recent findings raised doubts that IL-17A should still be considered an attractive target in MS: contradictory to previous findings, both overexpression and genetic deficiency or neutralization of IL-17A had no significant effect in EAE; in a clinical trial, treatment with anti-IL-12/23p40 antibody did not have a beneficial effect in MS, which came as a surprise since analogous approaches efficaciously suppressed EAE. Here we review findings about the role of IL-17A in EAE, with an emphasis on its potential as a therapeutic target in MS.

Keywords: IL-17A, multiple sclerosis, CNS.

INTRODUCTION

Multiple sclerosis (MS) and its animal model, experimental autoimmune encephalitis (EAE), were for two decades thought to be mediated by Th1 cells. However, IL-12 [1-3] and IFN-γ [4, 5], two hallmark Th1 cytokines, have been shown to play a suppressive role in EAE, casting doubt on the Th1 paradigm. In contrast to EAE, treatment with IFN-γ worsened MS [6, 7], raising the possibility that major immunological pathways that dominate pathogenesis of these diseases differ. Similarities of pathologic processes in EAE and MS are still being debated; nonetheless, EAE remains an essential tool for studying MS and discovering therapies. Findings that deficiency in IL-23 [8] confers protection from EAE and that IL-23 selectively expands highly encephalitogenic IL-17A-producing $CD4^+$ T cells [9] soon led to discovery of the Th17 lineage [10, 11], supplanting the previous view that a subset of memory Th1 cells produces IL-17A [12]. Discovery of the Th17 lineage marked the first major revision of the Th1/Th2 paradigm and instituted the Th17 model of EAE and MS pathogenesis. Previously puzzling suppressive effects of Th1 cytokines IFN-γ and IL-12 in EAE were easily explained by their potent inhibitory effects on Th17 cells [10, 11, 13], which were readily declared the main culprits in autoimmune CNS inflammation. The Th17 model of MS/EAE in its extreme form presumes that the importance of the IL-23/Th17 axis completely negates the formerly favored Th1 model and that Th1 cells and cytokines might even be beneficial due to the suppression of, or competition with, Th17 cells. However, a number of studies have demonstrated that IL-12 [14] directly promotes encephalitogenicity of myelin-specific Th1 cells in adoptive EAE. Furthermore, injection of IL-12 promotes relapse in the relapsing-remitting EAE model [15] and induces relapse in otherwise monophasic EAE [16]. These and similar findings are frequently cited to support a moderate view that both Th1 and Th17 cells are capable of mediating similar clinical syndromes, but likely *via* distinct inflammatory pathways [14]. Thus, both Th1 and Th17 cells can be pathogenic and the view has been promoted that the role of one lineage over another should not be considered more important [17]. Demonstrations that Th1 cells and cytokines can induce or promote disease under certain experimental conditions may provide an indication but do not prove that they have such a role in a natural disease pathogenesis. This can be illustrated by the finding that Th2-polarized myelin-specific cells can induce EAE upon adoptive transfer [18]; however, it is not believed that Th2 cells play a pathologic role in natural development of EAE and MS. Experimental evidence to date clearly demonstrates that all factors which directly impair development of Th17 cells (type I IFNs, IFN-γ, IL-12, IL-27) or deficiency of factors that promote this lineage (IL-1, IL-6, IL-23) consistently either ameliorate or abrogate EAE. In contrast, factors that promote Th1 cell development or mediate their function (IL-12, IFN-γ) are highly suppressive in EAE. Nevertheless, it is undeniable that during EAE induction Th1 cells constitute a large portion of anti-myelin response and that these cells can be pathogenic as well. An additional layer of complexity is added by the apparent plasticity of the Th17 phenotype. IL-

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1876-8946/10

$17A^+IFN\text{-}\gamma^-$ Th17 cells readily convert to $IL\text{-}17A^+IFN\text{-}\gamma^+$ Th1/Th17 and $IL\text{-}17A^-IFN\text{-}\gamma^+$ Th1 cells [13, 19, 20], precluding a clear view of the roles that Th1 and Th17 lineages play in EAE pathogenesis. Considerations on the role of different lineages in EAE have relevance for identifying and prioritizing therapeutic targets in MS. Most of the knowledge on this topic comes from animal studies and might not be applicable to MS. Perhaps the most obvious example of dissimilarities is the above-mentioned worsening of MS caused by IFN-γ treatment, an outcome opposite to that in EAE [6, 7]. Potentially, EAE and MS share similar pathologic pathways but are often studied at disease stages that are not equivalent. EAE has an acute inflammatory nature and is most frequently studied during the first several weeks post immunization, while studies in MS and clinical trials are typically conducted in patients who are in a chronic phase of disease. Hypothetically, mechanisms of disease initiation and progression differ, with factors that dominate early disease and those that drive the chronic phase being distinct. A similar view has been put forward to explain the failure of anti-IL-12/23p40 treatment in a MS clinical trial] [21]. The biggest surprise was that neutralization of IL-23 did not have a therapeutic effect, a result that indicated the insignificant role of Th17 cells in MS, and also of IL-17A by association. However, it should be borne in mind that IL-12 was blocked as well, thus, by the same logic, making Th1 cells also irrelevant in MS.

This review compares published data regarding the role of IL-17A in EAE and contrasts pro and con arguments for targeting IL-17A in MS. We focus on IL-17A because no other cytokine typically associated with Th17 cells (IL-17F, IL-21, IL-22) contributes to their encephalitogenicity. In addition to IL-17A we briefly consider IL-17F because of dimer formation with IL-17A and shared receptor subunits.

DISCUSSION

IL-17A and IL-17F

IL-17A (also known as IL-17) was first isolated from an activated mouse T-cell hybridoma in 1993 by Rouvier *et al.*, as a cDNA transcript termed CTLA8 [22], and a human IL-17A homologue was also identified as a T cell-derived factor with cytokine activity [23]. IL-17A became the founding member of the IL-17 family of cytokines that now has 6 members (IL-17A-F) in both humans and mice [24]. The sixth member of the family, IL-17F, is located adjacent to IL-17 in human and mouse genomic sequence [25, 26]. IL-17A and IL-17F are the most similar, with 50% identity in amino acid sequence, while IL-17E (also called IL-25) is most divergent in the family. IL-17F is the only member of the family whose 3-dimensional crystal structure has been solved [25]. It forms a cysteine knot structure also found in the NGF and PDGF cytokines, and the conserved cysteine alignment and computer modeling of IL-17A indicate that it almost certainly adopts a similar structure. Comparison of human IL-17s with other species suggests that IL-17 family members are highly evolutionarily conserved. Members of the IL-17 family are all expressed as dimers, and with the exception of IL-17B, they are covalent dimers [24]. IL-17A and IL-17F exist as homodimers, and it was recently shown that both in mouse and human systems, IL-17A and IL-17F are secreted in the form of disulfide linked heterodimers [27-29]. Thus, cells that express both cytokines, i.e. Th17 cells, in addition to IL-17A and IL-17F homodimers also secrete IL-17A/F heterodimers. Functionally, IL-17A/F heterodimers signal through the same receptor as IL-17A and IL-17F homodimers and induce similar biological responses. However, both mouse and human IL-17A demonstrate more than 100-fold higher biological activity than IL-17F, while IL-17A/F has intermediate activity.

Receptors for IL-17A and IL-17F

The IL-17 receptor (IL-17R or IL-17RA) is the cognate receptor for IL-17A, and it is widely expressed in various tissues and cell types [30]. When discovered, IL-17RA was structurally unique, but more recently four additional receptors (IL-17RB-F) related to IL-17RA have been discovered [31]. The ligand-receptor relationships are still poorly defined for this family. It was thought that IL-17RA was solely responsible for signaling of IL-17A and potentially IL-17F. However, recently IL-17RC has been identified as necessary for the biological effects of IL-17A and IL-17F [32-34]. The receptor for IL-17A seems to comprise two IL-17RA subunits and one IL-17RC subunit, though the precise stoichiometry remains unknown [32, 35]. IL-17RA binds both IL-17A and IL-17F, but it binds IL-17A with higher affinity [36]. IL-17RC is the cognate receptor for IL-17F [37]. While human IL-17RC also binds IL-17A, mouse IL-17RC appears to be specific for IL-17F and does not bind mouse IL-17A [37]. The human IL-17F/IL-17A heterodimeric cytokine signals through the IL-17RA/IL-17RC receptor complex [36]. IL-17RC exists in a large number of splice isoforms that differ in the extracellular domain [38, 39]. These splice forms may differ in their ability to bind ligands or associate with IL-17RA, potentially modifying signaling in target cells. A soluble form of IL-17RC effectively inhibits IL-17A and IL-17F signaling, suggesting that a soluble form of this molecule may serve as an effective therapeutic antagonist of IL-17A and IL-17F [37].

Cellular Sources of IL-17A and IL-17F

Th17 cells are frequently considered the primary source of IL-17A and IL-17F. However, a number of other cell types produce these cytokines: $CD8^+$ T cells [40], γδ T cells [41], NKT cells [42], NK cells [43], monocytes [26], microglia [44], neutrophils [45], and eosinophils [46], astrocytes and oligodendrocytes [47]. Thus, IL-17A and IL-17F are produced by cells of both the innate and the adaptive immune systems as well as non-immune cells. Unlike in most EAE models $CD8^+$ T cells may be pathogenic in MS, making it important to note that $CD8^+$ T cells can acquire a phenotype, Tc17, that parallels Th17 and secrete large quantities of IL-17A and IL-17F [40]. This is of particular interest since an abundance of $CD8^+$ T cells was found in acute MS lesions and a surprisingly large proportion (~80%) of them produced IL-17A [47].

Biological Effects of IL-17A and IL-17F

Similar stimuli induce IL-17A and IL-17F in $CD4^+$ T and $CD8^+$ T cells [40] and both cytokines have similar proinflammatory properties [31]. They induce the expression of proinflammatory cytokines (IL-6, IL-1β, TNF, GM-CSF, G-CSF), chemokines (CXCL1, CXCL8, CXCL10), and metalloproteinases in a broad spectrum of cells [48-53]. CCL20, a

ligand for CCR6 that has been linked to Th17 recruitment to inflamed sites including the CNS [54], is another IL-17 target gene. The role of CCL20/CCR6 in autoimmune CNS inflammation has lately been in focus and contradictory findings have been published by different groups. While some have found that CCR6 is required for EAE development [54, 55], others have reported that deficiency in CCR6 results in hypersusceptibility to EAE [56, 57]. Human Th17 cells are capable of producing CCL20 themselves [58]. IL-17A and IL-17F are key cytokines for the recruitment, activation, and migration of neutrophils [24, 38]. IL-17A appears to act on neutrophils indirectly, both through their expansion and recruitment [59]. In mice, the most relevant target chemokines seem to be CXCL1 and CXCL5 [49, 60, 61], while in humans expression of IL-8 is likely the most important neutrophil-attracting effect induced by IL-17A [51].

In the context of Th17-mediated immunity IL-6 is usually associated with development of this lineage. However, IL-6 is also a critical downstream target of IL-17A, where these two cytokines, in a positive-feedback loop, induce expression of IL-6 in fibroblast cells. Blockade of the IL-6 significantly suppresses the development of EAE, leading to the conclusion that IL-17A promotes EAE by triggering a positive-feedback loop *via* IL-6 induction from non-hematopoietic cells [62]. Taking into account the known inhibitory effect of IL-6 on development of regulatory T cells it has been proposed that IL-17A-induced expression of IL-6 in inflamed tissues or the peripheral lymphoid system suppresses the development of regulatory T cells, which in turn can promote Th1 responses [63]. An example of suppressive effects that IL-17A-induced IL-6 has on regulatory T cells comes from the model of nasal tolerance. During tolerance induction co-administration of IL-17A increased IL-6 release, promoted Th17 cell development and decreased the number of Treg cells, preventing tolerance induction and maintaining susceptibility to EAE [64].

It was generally believed that IL-17A does not act directly on $CD4^+$ T cells, but recent discoveries showed that IL-17A suppresses Th1 development in humans [65] and mice [66, 67] and even Th17 cells [68], adding T cells to the list of its cellular targets. Action on $CD4^+$ T cells may explain upregulation of IFN-γ production in mice deficient for IL-17A [69].

Th17 Cells in MS

It has become increasingly clear that Th17 cells are important in autoimmune inflammation [31]. Th17 cells have been shown to directly contribute to uveitis and scleritis in humans [70]. Elevated IL-17A has been detected in the synovial fluid of rheumatoid arthritis patients [12] and in brain lesions and cerebrospinal fluid of MS patients [71-74]. A study in an Asian population showed that IL-17A and IL-8 were significantly higher in opticospinal than in conventional MS patients [71]. The authors concluded that in opticospinal MS intrathecal activation of the IL-17A/IL-8 axis, which induces heavy neutrophil infiltration, contributes to extensive spinal cord lesion formation. Tzartos *et al*. showed expression of IL-17A in perivascular lymphocytes and, surprisingly, in astrocytes and oligodendrocytes located in active areas of MS lesions [47]. They report enrichment of IL-17A-producing $CD4^+$ and $CD8^+$ T cells (70-80 %) in active MS lesions, while only 7 % of lymph node T cells were IL-$17A^+$. Expression of IL-23 is higher in myeloid dendritic cells (DCs) from patients with MS than in healthy controls and DCs from such patients produce more IL-23, suggesting a mechanism for expanding Th17 cell populations in these patients [74]. Examination of brain tissue from patients with MS has shown expression of IL-23p19 in both active and chronic lesions [75], indicating that IL-23 may be pivotal in augmenting the inflammatory response in MS lesions and consequently increasing the potential for damage. These observations suggest an important role for IL-17A in MS pathogenesis [47]. Kebir *et al*. [76] showed the expression of IL-17A and IL-22 receptors on blood-brain barrier (BBB) endothelial cells in MS lesions, and that IL-17A and IL-22 disrupt BBB tight junctions *in vitro* and *in vivo*. Furthermore, Th17 lymphocytes efficiently migrate across the BBB, kill human neurons and promote CNS inflammation through $CD4^+$ lymphocyte recruitment [76].

The Role of IL-17A in EAE

Discovery of the Th17 lineage is intricately associated with EAE. A landmark article by Langrish *et al*. described potent encephalitogenicity of IL-23-polarized myelin-antigen specific IL-17A-producing $CD4^+$ T cells compared to Th1 cells [9]. Although the authors stopped short of declaring IL-$17A^+CD4^+$ cells a new Th lineage, similar to another group whose work preceded them [77], they set the stage for retirement of the 20 year-old Th1/Th2 paradigm of autoimmunity. Soon thereafter, Th17 cells were defined as a separate Th lineage [10, 11], which radically changed the landscape of autoimmunity research and therapy. Since Th17 cells seemed to be the principal culprits in autoimmune inflammation of the CNS it was plausible that their hallmark cytokine, IL-17A, mediates their pronounced encephalitogenicity. This possibility was supported by the already well-known pro-inflammatory effects of IL-17A and its disease-promoting role in collagen-induced arthritis (CIA) [78-80], a disease model whose pathogenesis often parallels EAE. As expected, the study that established high encephalitogenicity of Th17 cells also confirmed the important role of IL-17A in EAE pathogenesis [9]. This finding taken together with the preceding discovery that active MS lesions contain large quantities of IL-17A mRNA [72] initiated this cytokine into a circle of prime therapeutic targets for MS.

The first published notion that IL-17A plays a role in EAE came from Iwakura's group in 2003, in an article describing its role in CIA and also remarking on their unpublished data that EAE in IL-$17A^{-/-}$ mice was significantly suppressed [78]. An article focused on the role of IL-17A in EAE was published by the same group 3 years later [69] and they recapitulated their main findings in a subsequent publication [81]. Mice deficient in IL-17A exhibited delayed onset of disease, reduced maximum severity scores, ameliorated histological changes, and early recovery in EAE [69]. Paralleling their findings in CIA [78], T cell response against MOG_{35-55} was reduced in IL-$17A^{-/-}$ mice, suggesting a role of IL-17A in the priming phase of EAE in addition to the effector phase. Fractions of IFN-γ-producing $CD4^+$ and $CD8^+$ T cells specific for MOG_{35-55} were increased in IL-$17A^{-/-}$ mice, indicating that IL-17A negatively regulates the development of IFN-γ-producing Th1 cells. However, IL-17A did not affect IFN-γ production by $CD4^+$ T cells directly, indicating

that IL-17A suppresses Th1 indirectly, through its action on other cells. Surprisingly, levels of anti-MOG_{35-55} Abs were increased in IL-$17A^{-/-}$ mice compared to WT mice. This was especially pronounced during the chronic phase of EAE, although IL-$17A^{-/-}$ mice had fully recovered from EAE at that time. These results were unexpected because Ab production was suppressed in IL-$17A^{-/-}$ mice during CIA and contact, delayed-type, and airway hypersensitivity [78, 82]. Lack of correlation between increased anti-MOG_{35-55} Abs and clinical disease indicates the marginal role of Abs in this EAE model. The important role of T cell-derived IL-17A in development of EAE was confirmed when adoptively transferred IL-$17A^{-/-}$ $CD4^+$ T cells re-activated *in vitro*, without addition of exogenous cytokines, were inefficient in inducing EAE in recipient mice. However, reduced anti-MOG_{35-55} T cell response in IL-$17A^{-/-}$ mice, described in the same article [69], was not considered as a possible contributing factor to impaired encephalitogenicity of IL-$17A^{-/-}$ $CD4^+$ T cells. Another paper described a similar experiment where IL-$17A^{-/-}$ $CD4^+$ T cells obtained from mice immunized with MOG_{35-55} were reactivated *in vitro* in the presence of IL-23 and then transferred into WT recipients. Lack of IL-17A expression in Th17 cells resulted in attenuated clinical EAE compared to EAE induced by WT Th17 cells [62]. Even though potentially different magnitudes of anti-MOG_{35-55} responses between donor WT and IL-$17A^{-/-}$ mice were again not considered as a factor in observed differential encephalitogenicity, these data perhaps more closely reflect the contribution of IL-17A to the pathogenicity of Th17 cells in EAE, which is important but not an essential factor.

As mentioned above, the first data on the role of IL-17A in EAE were published by Langrish *et al*. in 2005. SJL mice were treated with a single injection of anti-IL-17A Ab seven days post immunization with $PLP_{139-151}$. Treatment markedly suppressed the first attack, albeit not completely [9]. Potentially, repeated injections of the Ab would have produced more pronounced suppression of disease. The experiment was terminated during the first attack, precluding insights into the consequences of early IL-17A neutralization on relapses. This finding is confirmed in a recent study where *in vivo* neutralization of IL-17A with Ab injected on days 6, 10 and 14 post immunization did not delay disease onset but significantly reduced the severity of clinical disease in SJL mice immunized with $PLP_{139-151}$ [83].

Adoptive EAE induced by transfer of IL-23-expanded Th17 cells was not suppressed by anti-IL-17A Ab treatment of recipient mice (data not shown) [9]. Nonetheless, these findings for the first time directly demonstrated that IL-17A significantly contributes to pathogenesis of EAE. However, incomplete protection from EAE when IL-17A was neutralized, in particular in adoptive EAE, demonstrated that IL-17A is not an essential factor in EAE pathogenesis as is IL-23. Later on, in a similar study treatment with anti-IL-17A Ab partially inhibited adoptive EAE in SJL mice induced by transfer of IL-23-polarized, but not IL-12-polarized, cells [14].

The observation that neutralization of IL-17A did not have an effect on adoptive EAE induced by Th1 cells is not surprising, given that these cells do not produce IL-17A. It should be noted that a fairly large number of Th1 cells ($25x10^6$) was transferred into recipient mice, potentially circumventing a need for other factors (i.e. IL-17A) otherwise involved in normal EAE induction. However, a study using a 5-10-fold lower Th1 cell number showed that IL-17A produced by recipient mouse cells contributes to disease in the adoptive EAE model. Transferred encephalitogenic Th1 cell line-induced recruitment of host IL-17A-producing αβ and γδT cells to the CNS during the initiation of EAE and these recruited cells contributed to the incidence and severity of disease [84]. Cells of recipient mice accumulated in the CNS within 3 days of adoptive transfer and played a role in EAE induction in an IL-17A-dependent manner given that transfer of Th1 cells into IL-17A-deficient recipients resulted in ameliorated EAE. This observation raises the question of the relative importance of different IL-17A cellular sources in EAE. Th17 cells are typically considered the most important source of this cytokine in EAE, while other cells are often overlooked. In particular, γδT cells that infiltrate the CNS are highly enriched in IL-17A producers, with this phenomenon occurring in both direct and adoptive EAE [84]. Unexpectedly, γδT cells appear to be responsible for much of the IL-17A produced in several disease models, especially early on [85]. Hypothetically, significant amelioration of EAE in γδT cell-deficient mice [86, 87] can in part be due to reduced IL-17A production.

Of particular interest in the evaluation of IL-17A as a therapeutic target in MS is that neutralization of IL-17A initiated after the first EAE attack in SJL mice completely prevented disease relapse and stopped its progression almost as efficiently as neutralization of IL-23 [88]. Anti-IL-17 treatment reduced expression of IL-1, IL-6, and CCL6, while blockade of IL-23 had a similar effect, but on a larger spectrum of proinflammatory mediators. Even though neutralization of IL-17A blocked EAE relapse, it did not significantly reduce the number of infiltration foci, suggesting that IL-17A likely downregulates the effector function of inflammatory cells within the CNS rather than altering inflammatory cell migration and/or expansion. These findings demonstrated the crucial role of IL-17A in the pathogenesis of EAE relapse and affirmed IL-17A as an attractive therapeutic target in MS. This view was supported by a study using vaccination against IL-17A. Immunization of mice with IL-17A-OVA complexes induced production of endogenous neutralizing Ab that completely prevented PLP-induced EAE in SJL mice. However, vaccination seems not to have affected anti-PLP responses, indicating that IL-17A plays a role solely in the effector phase of EAE. In the same study treatment with monoclonal anti-IL-17A Ab also prevented EAE in SJL mice [89]. In a subsequent study, the same researchers also showed complete protection of C57BL/6 mice from development of EAE and inflammation of the CNS [90]. Another study that demonstrated the almost absolute requirement for IL-17A in EAE showed that the blockade of IL-17A by injections of neutralizing Ab throughout the experiment completely prevented EAE [91]. In this study $ICOS^{-/-}$ mice developed more severe EAE and expressed higher levels of IL-17A, IL-6, and TGF-β mRNA in the spinal cords at the onset of the disease than WT animals. Treatment with Ab strongly inhibited disease even in hypersusceptible $ICOS^{-/-}$ mice, showing that IL-17A plays a major role in the pathogenesis of EAE [91].

The laboratory of Dong C., simultaneously with another group [11], defined Th17 cells as a novel lineage [10] and

was among the first to study the role of IL-17A in autoimmune inflammation of the CNS. Treatment of C57BL/6 mice with anti-IL-17A Ab starting on day 9 post immunization with MOG_{35-55} and then every other day for a total of three injections resulted in considerably delayed onset of disease. Mice treated with anti-IL-17A Ab developed disease 6 days after the last Ab injection. Perhaps if Ab administration had been continued disease would not have manifested at all. Consistent with the absence of clinical signs there was no obvious cellular infiltration in the CNS during the treatment with anti-IL-17A Ab. Splenocytes from mice treated with anti-IL-17A Ab proliferated normally upon stimulation with MOG_{35-55}, and expression of proinflammatory cytokines TNF-α, IFN-γ and IL-17A was not inhibited by the Ab treatment, demonstrating that blocking IL-17A in the effector phase of EAE does not suppress autoreactive T cells. Anti-IL-17A Ab treatment post disease onset reversed the progression of EAE and reduced numbers of $CD4^+$ T cells and $CD11b^+$ macrophages in the CNS [10]. The same group later generated IL-$17A^{-/-}$ mice and found significantly delayed disease onset, markedly reduced severity and progression compared to WT mice, demonstrating that IL-17A has non-redundant function in autoimmune CNS inflammation. Among infiltrating mononuclear cells in the CNS, $CD4^+$ T cells were greatly reduced in IL-$17A^{-/-}$ mice compared to WT mice. IL-17A deficiency caused greatly elevated IFN-γ production by splenocytes stimulated with MOG_{35-55} compared to WT cells [92].

BALB/c mice are largely resistant to EAE induction. However, BALB/c mice deficient in both STAT6 and T-bet ($STAT6^{-/-}$T-$bet^{-/-}$), and hence incapable of mounting Th1 and Th2 responses, developed severe chronic EAE upon immunization with MBP [93]. This is surprising since several groups have shown that T-bet deficiency confers resistance to EAE [94, 95], including EAE in BALB/c mice [96], and that T-bet is necessary for encephalitogenicity of both Th1 and Th17 cells [97]. Nonetheless, EAE in $STAT6^{-/-}$T-$bet^{-/-}$ BALB/c mice was mediated by a robust Th17 response to MBP. Neutralization of IL-17A by Ab injections markedly delayed disease onset, reduced disease severity and led to early and complete recovery from disease, recapitulating earlier observations made in the C57BL/6 EAE model. This result reaffirms that IL-17A significantly contributes to EAE pathogenesis and encephalitogenicity of Th17 cells, although other cellular sources of IL-17A cannot formally be excluded. Since EAE in $STAT6^{-/-}$T-$bet^{-/-}$ mice develops without involvement of Th1 cells, and IL-17A neutralization has an effect highly similar to that observed in mice with strong anti-myelin Th1 responses, these findings again raise a question about the contribution of Th1 cells to EAE development and/or progression.

One study examined the role of IL-17RA in EAE using receptor-deficient mice [63]. Incidence of disease was greatly reduced (~20%) as well as peak clinical scores compared with WT mice. In mice that developed disease, onset was delayed and recovery sped up, showing that IL-17RA signaling is important for initiating, as well as sustaining EAE. Consistent with mitigated disease course, a significantly reduced number of $CD4^+$ T cells were harvested from the CNS of IL-$17RA^{-/-}$ mice compared with those from WT mice. Development of Th17 cells was normal in IL-$17RA^{-/-}$ mice and T cell proliferative responses to MOG_{35-55} were not impaired in IL-$17RA^{-/-}$ mice compared with controls. Surprisingly, the number of MOG-specific Th1 cells was drastically reduced in IL-$17RA^{-/-}$ mice, indicating that IL-17A signaling is required for the generation of MOG-specific Th1 cells. A few macrophages were observed in the spinal cord of IL-$17RA^{-/-}$ mice and the infiltration was in meninges instead of parenchyma as in the case of WT mice. Overall, this study showed that mice lacking IL-17RA were largely resistant to EAE, similar to most studies relying on mice deficient for IL-17A and supporting the view that this cytokine plays an important role in EAE.

The above-mentioned studies have somewhat dissimilar findings on the extent to which IL-17A influences EAE, its role ranging from being absolutely required to being important but not an essential factor in EAE development. Nonetheless, they all agree that IL-17A significantly worsens both chronic and relapsing-remitting EAE and that IL-17A plays a role in both the priming and effector phase of disease pathogenesis. In contrast, the following two studies deny that IL-17A significantly contributes to EAE, introducing controversy over whether this cytokine should be a therapeutic target in MS. In one of these studies neutralization of IL-17A with Ab before onset of EAE, induced in C57BL/6 mice with MOG_{35-55} peptide, modestly attenuated its clinical course, and neutralization of IL-17A with IL-17-receptor-Fc-fusion protein after disease onset only marginally improved clinical signs [98]. Overall reduction in EAE severity was minimal, indicating redundancy of IL-17A in EAE.

In a most recent and more extensive study Haak *et al*. generated mice whose T cells constitutively express IL-17A in the steady state [99]. Expression of IL-17A by T cells did not have obvious effects on the immune system judged by the composition of thymus and spleen and production of other cytokines such as IFN-γ, IL-4 and IL-2. Unexpectedly, immunization of these mice with MOG_{35-55} did not result in more severe clinical EAE and inflammation of the CNS or altered levels of IFN-γ, IL-6 and GM-CSF. Thus, increased production of IL-17A by T cells did not exacerbate EAE. To further explore the role of IL-17A in EAE they used IL-$17A^{-/-}$ mice generated by Iwakura's group. However, in contrast to observation of Iwakura's lab [69] these mice developed an insignificant decrease in disease severity and incidence and minimal but significant difference in day of onset, indicating the non-essential role of IL-17A in EAE. Information on whether IL-17A-deficiency had an effect on the magnitude and quality of anti-MOG_{35-55} response in this study is lacking. To eliminate potential compensatory effects of IL-17F, they treated IL-$17F^{-/-}$ mice with neutralizing anti-IL-17A Ab and again found only a minimal beneficial impact on disease development. The authors therefore concluded that IL-17A, while prominently expressed by an encephalitogenic T cell population, may only marginally contribute to EAE.

Overall findings on the role of IL-17A in EAE are highly similar to those made in some other models of organ-specific autoimmunity. In the experimental autoimmune uveitis (EAU) model neutralization of IL-17A during the entire course of the experiment or starting at the effector phase of disease was highly protective [100]. The magnitude of immune response, including DTH, proliferation, and cytokine production, was strongly reduced in both treatment regimens. Similar conclusions were reached by another group,

who also found that blockade of endogenous IL-17A by treatment with anti-IL-17 antibody attenuates EAU in rats [101]. Unlike in the case of IL-17A, neutralization of IL-23 did not inhibit disease when treatment was started from day 7 after immunization [100]. This is similar to EAE, where it has been shown that IL-23 is essential for terminal differentiation of Th17 cells and that neutralization of IL-23 after the priming phase does not prevent disease [102]. As in EAE, neutralization of IL-17A had no effect on EAU when disease was induced by adoptively transferred uveitogenic Th1 cells [100]. Interestingly, inhibition of EAU in IL-17A$^{-/-}$ mice was only partial and did not attain statistical significance. An apparent contrast between the protective effect of IL-17A neutralization in WT mice and susceptibility of IL-17$^{-/-}$ mice to EAU was interpreted as genetic compensation, a phenomenon not uncommon in knockout mice [103].

The Role of IL-17F in EAE

In most studies, neutralization of, or genetic deficiency in, IL-17A suppressed EAE incompletely. Since both IL-17A and IL-17F are produced by Th17 cells and both cytokines have similar biological effects, it seemed plausible that IL-17F partially compensates for the lack of IL-17A and mediates residual EAE susceptibility. However, in contrast to IL-17A, all the studies that investigated the role of IL-17F in EAE consistently found that this cytokine does not play a role in EAE, and does not have additive, synergistic, or compensatory effects to those of IL-17A [81, 92, 99]. Even though lack of IL-17F did not impact clinical EAE, in one study IL-17F$^{-/-}$ mice exhibited somewhat improved recovery in comparison to WT animals [92]. Surprisingly, examination of the CNS inflammatory infiltrate showed greatly reduced CD4^{+} T cell numbers in both IL-17F$^{-/-}$ and IL-17A$^{-/-}$ mice compared to WT mice, whereas CD11b^{+} cells were slightly increased in IL-17F$^{-/-}$ animals. Expression of CCL2 and CCL7 was greatly reduced in the CNS of IL-17F$^{-/-}$ compared to WT mice, and even more profoundly in IL-17A$^{-/-}$ mice, while CXCL1 expression was impaired only in IL-17A$^{-/-}$ animals [92]. Differences in expression of the above chemokines potentially can account for dissimilar composition of CNS infiltrates between WT and knockout mice. However, it is surprising that those differences did not translate into different clinical courses between IL-17F and WT animals. Largely unaltered clinical EAE in IL-17F$^{-/-}$ mice is in agreement with unpublished data of the same group that treatment of WT mice with anti-IL-17F Ab does not affect clinical EAE [92].

Contrasting the above findings, another group did not detect differences in cell number and composition of CNS-infiltrating cells between IL-17F$^{-/-}$ and WT animals, in agreement with normal development of autoreactive T cells and unaltered susceptibility to EAE [99]. Treatment of IL-17F$^{-/-}$ mice with neutralizing anti-IL-17A Ab had only a minimal inhibitory effect on clinical disease, eliminating potential compensatory effects of this cytokine.

Iwakura's group also showed that IL-17F$^{-/-}$ mice develop EAE indistinguishable from WT mice, while IL-17F$^{-/-}$IL-17A$^{-/-}$ double knockout mice develop suppressed EAE with a clinical course identical to that in IL-17A$^{-/-}$ mice [81].

Results from these studies on IL-17F have indicated that this cytokine might positively regulate IL-17A production, but the extent of IL-17A suppression due to the lack of IL-17F does not appear to be sufficiently severe to influence EAE development.

The role of IL-17A in EAE is currently controversial, with findings ranging from the complete dispensability [99] of IL-17A to its being an absolute requirement [89] in the pathogenesis of EAE. Nonetheless, most researchers found that IL-17A contributes significantly to the development of EAE, even if it is not essential for manifestation of disease. In most cases genetic deficiency in, or neutralization of, IL-17A led to delayed disease onset, reduced severity of clinical signs and improved recovery from the disease. This mitigated disease course was typically accompanied by a reduced amount of cellular inflammatory infiltrates in the CNS. It is puzzling that different groups, using similar approaches, obtained discrepant data. Certain contradictory observations can potentially be due to differences in experimental design, such as various dosing regimens of neutralizing anti-IL-17A Ab or variations in protocols for adoptive EAE induction. However, differences between various laboratories using IL-17A$^{-/-}$ mice are less likely to be caused by variability in experimental technique.

In this review we have primarily focused on the role that IL-17A has in clinical EAE, as the most important readout that sums up its functions in the priming and effector phases of EAE. A comprehensive review of the effects of IL-17A on ensuing immune responses is beyond the scope of this review, but a brief comparison of findings from different groups shows the same inconsistency observed in clinical EAE. Some researchers found that IL-17A had no effect on development of anti-myelin antigen responses as determined by their magnitude and quality. In contrast, other researchers found that IL-17A promotes immune responses with or without concomitant suppression of Th1 responses and IFN-γ production. Contradictory findings prevent clear conclusions on the extent to which the overall role of IL-17A in EAE is determined during the priming versus effector phase. Nevertheless, it appears that the most consequential effects of IL-17A are those that occur during the effector phase of disease.

The majority of research on IL-17A has been done in C57BL/6 mice, and 2 groups independently generated IL-17A$^{-/-}$ strains on this background [69, 92]. The other 2 mouse models that have been used for testing the role of IL-17A in EAE were in SJL and STAT6$^{-/-}$T-bet$^{-/-}$ BALB/c mice using the Ab neutralization approach. Findings in the $PLP_{139-155}$/SJL model have been more consistent than in the MOG_{35-55}/C57BL/6 model, but it is possible that consistency might be due to the limited number of studies and available approaches. Nonetheless, in agreement with most studies in C57BL/6 mice, IL-17A has been shown to play an important role in EAE pathogenesis in SJL mice, including relapse. The low level of IL-17RA expression in the CNS was proposed as an explanation for the lack of effects of IL-17A in EAE by the authors who did not observe a significant contribution of IL-17A to EAE in C57BL/6 mice [99]. A corollary of this argument is that SJL mice express a higher level of IL-17RA in the CNS since EAE in this strain seems to be more consistently impacted by IL-17A than in C57BL/6 mice. A study comparing expression of IL-17RA in the CNS between different mouse strains has not been published, but

the possibility that SJL and C57BL/6 mice express markedly different levels of this receptor in the CNS seems unlikely. If there are differences in the contribution of IL-17A to autoimmune CNS inflammation in various mouse strains it is likely that they are caused by dissimilarities in the relative importance of various pathways in disease pathogenesis. This can be exemplified by the finding that B7.1 and B7.2 deficiency caused resistance to EAE in C57BL/6 but not SJL mice, demonstrating that genetic background determines the relative importance of various pathways in EAE development [104].

Th17 cells that develop under the influence of TGF-β and IL-6 without subsequent exposure to IL-23 produce large quantities of IL-17A but are non-encephalitogenic upon adoptive transfer, even when injected directly into the CNS [105]. Dissection of the effects of IL-23 on Th17 cells showed that it is required for terminal differentiation of Th17 cells and acquisition of pathogenic phenotype [102]. These findings demonstrated that the potential of T cells to produce IL-17A is in itself not sufficient for their encephalitogenicity, which is sometimes used as an argument to support the view that IL-17A does not play a role in EAE. However, this finding only demonstrates that the capability of T cells to produce IL-17A does not in itself confer encephalitogenicity, just as the lack of IL-17A production by Th1 cells does not prevent them from inducing adoptive EAE. Neither of these two observations is a valid argument for excluding IL-17A as a contributing factor in natural EAE development; nor are these observations valid arguments for the exclusion of Th17 cells.

CONCLUSION

A genetic deficiency in IL-23 prevents EAE [8] and its neutralization abrogates relapses [9]. These key roles of IL-23 in the development and progression of autoimmune inflammation of the CNS made IL-23 a prime therapeutic target in MS. Surprisingly, neutralization of IL-23 with anti-IL-12/23p40 Ab (ustekinumab) resulted in no clinical or radiologic improvement in MS patients [15]. The inefficiency of anti-p40 Ab has been explained by an inadequate choice of patient cohort; in particular, a large proportion of patients entered in the clinical trial had been diagnosed with MS for years [21]. Studies in mice have shown that IL-23 primarily influences development of encephalitogenic immune responses and that its role in the effector phase of disease is not as crucial [102, 106]. Based on these observations, it has been suggested that blocking of IL-23 in recently diagnosed MS patients, or those that have experienced only one attack (clinically isolated syndrome), with possibly still developing autoimmune responses, is more likely to be efficient than in long-term MS patients with advanced disease [21]. Although IL-23 has been shown to be most important during development of myelin-specific Th17 responses this view overlooks data showing that blockade of IL-23 started after the first attack of relapsing-remitting EAE in SJL mice completely prevented relapse [88]. Nonetheless, failure of anti-IL-23 therapy in a clinical trial has dampened enthusiasm for further pursuit of IL-23 as a therapeutic target in MS. The role of IL-17A in EAE, or more precisely, the extent to which IL-17A contributes to disease is still controversial. This uncertainty will perhaps prevent or delay testing the therapeutic efficacy of the anti-IL-17A Ab approach in MS. Despite disparate conclusions about whether IL-17A "essentially" or "marginally" contributes to EAE, it is crucial to note that no findings indicate that blockade of IL-17A worsens disease, which is of utmost importance when considering treatment of MS patients with anti-IL-17A Ab. Probably the most relevant findings that indicate the potential for success of anti-IL-17A Ab therapy in MS are: 1) The majority of studies found marked suppression of disease when IL-17A was either absent or blocked, and blockade of IL-17A after the first attack efficiently prevented relapses and disease progression; 2) The role of IL-17A in MS is unknown and can only be inferred from animal studies. However, the extremely high enrichment of IL-17A-producing cells in active MS lesions indicates that neutralization of this cytokine has the potential of being beneficial. Anti-IL-17A monoclonal Abs (i.e. AIN457, Novartis) currently tested for treatment of psoriasis, noninfectious uveitis, Crohn's disease, and ankylosing spondylitis are being developed [107]. Perhaps they will one day be tested in MS as well.

ABBREVIATION

Ab	=	Antibody
BBB	=	Blood brain barier
CIA	=	Collagen induced arthritis
CNS	=	Central nervous system
DTH	=	Delayed type hypersensitivity
EAE	=	Experimental autoimmune encephalitis
EAU	=	Experimental autoimmune uveitis
G-CSF	=	granulocyte – colony stimulating factor
GM-CSF	=	granulocyte monocyte – colony stimulating factor
IL	=	Interleukin
IL-17R	=	Interleukin 17 receptor
IFN-γ	=	Interferon gamma
MOG	=	Myelin oligodendrocyte glycoprotein
mRNA	=	Messenger ribonucleic acid
MS	=	Multiple sclerosis
OVA	=	Ovalbumin
PLP	=	Proteolipid protein
TGF	=	Tumor growth factor
Th	=	T helper
TNF	=	Tumor necrosis factor
WT	=	wild type

ACKNOWLEDGEMENT

We are very grateful to K. Regan for editing the manuscript.

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Received: September 21, 2009 Revised: October 26, 2009 Accepted: October 28, 2009