Published in final edited form as:
Curr Opin Immunol. 2011 December ; 23(6): 707–712. doi:10.1016/j.coi.2011.08.005.

T_H17 cytokines in autoimmune neuro-inflammation

Burkhard Becher[1] and **Benjamin M Segal**[2]

[1]Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland [2]Holtom-Garrett Program in Neuroimmunology and Multiple Sclerosis Center, Department of Neurology, University of Michigan, Ann Arbor, MI 48109, USA

Abstract

It has been firmly established that IL-23 polarized T_H17 cells are potent effectors in the pathogenesis of experimental autoimmune encephalitomyelitis (EAE). However, the relative importance of these cells in comparison to other encephalitogenic T_H subsets, and the mechanisms that they employ to effect inflammatory demyelination, are topics of continuing investigation. Interestingly, deletion of individual 'T_H17 cytokines', such as IL-17A, IL-17F, IL-22 and IL-21, does not phenocopy the complete EAE-resistance of IL-23-deficient mice. The instability of T_H17 cells *in vivo* introduces an additional layer of complexity to their role in the context of relapsing or chronic disease. Recent data indicate that IL-23 drives the production of myeloid activating factors, such as GM-CSF, by myelin-reactive T cells and facilitates their accumulation in the CNS. This review discusses the above issues in relation to the use of T_H17 cells and related factors as potential therapeutic targets and biomarkers in CNS autoimmune diseases such as multiple sclerosis (MS).

Introduction

Ever since the discovery that $CD4^+$ T helper (T_H) cells could be classified into subsets based on the cytokines they produce, it has become evident that *in vivo* pathogenic as well as protective immune responses tend to be skewed towards specific T_H lineages [1]. The nature of the T_H cell polarization pattern underlying MS has been the focus of intense research for 20 years, driven in large part by the quest to develop better drugs to treat that disorder. Hence, therapeutic agents that target a dominant encephalitogenic T_H subset or its products could retain the efficacy of more globally immunosuppressive agents while minimizing the risk of opportunistic infections and secondary neoplasia. Although T_H1 cells were initially thought to mediate inflammatory demyelination in MS, the discovery of T_H17 cells has forced a reexamination of that dogma [2, 3]. Recent data generated by studies in MS and in its animal model EAE, suggest that the mechanism of action of T_H17 cells in CNS autoimmunity is more complex than previously appreciated. Here we will discuss apparent paradoxes regarding the respective roles of T_H17 cells and their signature cytokines, IL-17A, IL-17 IL-22, IL-21, in the pathogenesis of autoimmune demyelinating disease in mice and men.

The role of T_H17 cells in EAE

For many years, T_H1 cells were deemed responsible for the initiation of autoimmune demyelination. Conversely, T_H2 cells were believed to have regulatory properties, leading

Corresponding authors: Becher, Burkhard (becher@immunology.uzh.ch) and Segal, Benjamin M (bmsegal@umich.edu).

some to propose 'immune deviation' from a T_H1 to a T_H2 response as a promising therapeutic strategy in MS [4]. This paradigm has since been challenged by the results of experiments with genetically engineered mice. Against expectations, mice deficient in IFNγ, IFNγ receptor or IL-18 succumb to EAE, while IL-4 deficient mice exhibit a clinical course comparable to that of wildtype controls [5–8]. The T_H1 polarizing factor, IL-12 is composed of covalently bound p40 and p35 chains. Paradoxically, while mice deficient in the p40 chain are resistant to EAE [9], those deficient in the p35 chain are not [10••, 11]. These apparent discrepancies were resolved by the discovery of IL-23, a heterodimer composed of the IL-12 p40 chain complexed with a unique p19 chain [12]. IL-23 p19 deficient mice phenocopy IL-12p40 deficient mice in their complete resistance to EAE [13••], underscoring the importance of IL-23, as opposed to IL-12 or IFNγ, in EAE pathogenesis [14]. IL-17A (IL-17) was soon identified as one of the cytokines produced by T cells in response to IL-23 [15]. The increased frequency of IL-17 secreting cells in myelin immunized mice led to the conjecture that they constitute the critical effector population in autoimmune demyelinating disease. This was supported by the demonstration that IL-23 polarized, IL-17 secreting myelin-reactive $CD4^+$ T cells are highly encephalitogenic following transfer into naïve syngeneic recipients [15, 16••, 17].

More recent experiments have indicated that the encephalitogenicity of IL-23 polarized cells is not solely attributable to the production of IL-17 itself or of other cytokines traditionally ascribed to the T_H17 panel. The course of EAE is unperturbed, or only ameliorated, in IL-17A knock-out mice and in wild-type mice treated with neutralizing antibodies specific for IL-17A [18•, 19]. Conversely, ectopic expression of IL-17A did not worsen EAE [20]. Genetic targeting of other T_H17 associated cytokines, such as IL-17F [20] or IL-22 [21], also failed to confer resistance to the disease. IL-21 was initially claimed to be a vital encephalitogenicity gene [22], but this finding was not reproduced by others [23, 24]. The dispensability of IL-17A is not explained by redundant activities of IL-17F, since mice treated with an IL-17 receptor-Fc fusion protein, that blocks both cytokines, only had a modest impact [19]. Furthermore, IL-17F knock-out mice succumb to EAE, despite treatment with an IL-17A neutralizing antibody, – albeit disease severity is reduced [20].

Based on the above discussion, it could be argued that it is more appropriate to view IL-17 as a surrogate marker of IL-23 modulation, rather than as a master effector cytokine, in the context of CNS autoimmunity [25]. This does not mean that IL-17 is completely irrelevant to the pathogenesis of inflammatory demyelinating disorders. Several lines of evidence indicate that, in certain instances, IL-17 facilitates leukocyte trafficking across the blood–brain barrier (BBB) and infiltration within the CNS. In some EAE models, CNS infiltrates induced by myelin-reactive T_H1 cells are confined to the subpial white matter, whereas those induced by T_H17 cells reactive to the same epitope extend into the parenchyma in finger-like projections [16••]. Kebir and colleagues reported that human T_H17 cells migrate across BBB endothelial cell monolayers more efficiently than T_H1 cells and that IL-17 and IL-22 directly stimulate CNS endothelial cells to express chemokines and downregulate tight junction proteins [26•]. IL-17 also stimulates astrocytes to produce ELR^+ CXC chemokines that could, in turn, attract neutrophils to the BBB and activate them to release vasoactive substances [27]. Leukocyte infiltration of the brainstem, as opposed to the spinal cord, appears to be particularly dependent on IL-17 and impeded by IFNγ [6, 17, 28•, 29]. The biological basis for this regional difference in IL-17 dependence is unknown.

IL-17 independent mechanisms of action of IL-23 in CNS autoimmunity

The literature summarized in the former section suggests that IL-23 promotes EAE by IL-17 independent, as well as dependent, pathways in a context-dependent manner. T_H17 cells have been described to develop *in vitro* after exposure to IL-6 and TGFβ [[reviewed in 30]],

but this cytokine cocktail does not generate an encephalitogenic population [31••]; pathogenicity is dependent on IL-23 and not on the secretion of IL-17 by TGFβ/IL-6 manipulated T cells [31••]. Two independent reports showed that, while IL-23 receptor signaling is not required for the activation and expansion of myelin-reactive T cells *in vivo*, it is required for their accumulation in the CNS [31••, 32]. It remains to be demonstrated whether IL-23 directly facilitates T cell trafficking to the CNS via upregulation of chemokine receptors and adhesion molecules and/or promotes T cell survival/retention once they have crossed the BBB. IL-23 polarized, myelin-reactive T cells induce EAE after a shorter latency than IL-12 polarized effectors [16••, 33]. Some have speculated that T_H17 cells initially access the CNS and create a microenvironment conducive to the subsequent entry of T_H1 cells [33]. This could explain why T_H1 cells do not cause EAE in actively immunized IL-23p19 deficient mice [13••]. However others obtained data proposing the opposite, namely that T_H1 cells enter before T_H17 cells [34].

Although IL-17A is largely dispensable for EAE development, there is growing evidence that other soluble factors produced by IL-23 polarized T cells play non-redundant roles in the development of demyelinating lesions. Granulocyte monocyte colony stimulating factor (GM-CSF) has been identified as an IL-23 driven cytokine [35••, 36••]. Furthermore, GM-CSF might enhance IL-23 production by antigen presenting cells, thereby forming a positive feedback loop [36••]. Autoreactive helper T cells specifically lacking GM-CSF fail to initiate neuro-inflammation despite expression of IL-17A or IFNγ, whereas GM-CSF secretion by IFN$\gamma^{-/-}$ IL-17A$^{-/-}$ helper T cells is sufficient to induce EAE [36••]. A critical role for GM-CSF in EAE was originally demonstrated by Bernard and colleagues [37]. They found that the resistance of GM-CSF deficient mice to EAE could be overcome by repeated systemic injections of recombinant GM-CSF following active immunization with myelin antigens. It was subsequently shown that the administration of recombinant GM-CSF triggers the mobilization of CD11b$^+$Ly-6C^{hi} monocytes into the circulation of those mice [38••]. These immature monocytes migrate to the CNS during EAE and acquire characteristics of dendritic cells by upregulating CD11c and MHC Class II *in situ* [38••, 39]. CNS CD11c$^+$CD11b$^+$ cells have potent antigen presenting capacities [40] and have been implicated in epitope spreading [41]. Consistent with these findings, bone marrow chimera experiments have pinpointed peripheral myeloid cells, as opposed to resident microglia, as the key GM-CSF responder population in EAE [36••]. A parallel mechanism by which GM-CSF potentiates EAE was revealed by the discovery that GM-CSF dependent CD103$^+$ dermal dendritic cells are superior to other dendritic cell subsets in activating encephalitogenic T cells in the periphery [42]. GM-CSF is the only known T cell-derived cytokine which has a non-redundant function in EAE, and thus represents the first identified IL-23-driven encephalitogenic T cell cytokine.

The plasticity of T_H17 cells and implications regarding their mechanism of action in EAE

The quest to define T_H17 cells more accurately and to pinpoint those characteristics responsible for their pathogenicity in autoimmune disease is complicated by the plasticity of the T_H17 'lineage'. Human, as well as murine, T_H17 polarized cells can upregulate T-bet and IFNγ and downregulate IL-17 after activation with Th1 polarizing factors *in vitro* [43••, 44]. A similar phenomenon has been observed *in vivo* [45•, 46]. Using IL-17A reporter mice, Hirota and colleagues recently showed that up to two thirds of CNS-infiltrating T_H17 cells (defined as CD4$^+$ T cells that express IL-17A and/or have expressed IL-17A at any point in the past) express the T_H1 signature cytokine, IFNγ in C57BL/6 mice with $MOG_{35–55}$ induced EAE [45•, 47•]. These so called 'ex-T_H17' cells could easily have been mistaken for 'authentic' T_H1 cells in previous studies, particularly since they turn off expression of IL-17A and RORγt and upregulate the T_H1 associated IL-12 receptor γ2

chain. The ability of encephalitogenic Th17 cells to lose IL-17 expression and upregulate IFNγ *in vivo* was originally shown in an independent study using IL-17F reporter mice [47•]. Interestingly, Hirota *et al.* found that the conversion of T_H17 cells into IFNγ$^+$ IL-17$^-$ cells was IL-23 dependent [45•].This raises the question of whether the acquisition of T_H1-like characteristics and encephalitogenicity by ex-T_H17 cells are causally linked. Kinetic studies indicate that T_H17 cells pass through an IL-17A/IFN γ double positive stage during their transition into ex-T_H17 cells[45•]. The presence of IL-17A/IFNγ double positive CD4$^+$ T cells in perivascular infiltrates and the adjacent white matter of CNS tissues from patients with MS suggest that ex-T_H17 cells might play a prominent role in the human disease as well [48]. Furthermore, a significant percentage of CD4$^+$CD45RO$^+$ lymphocytes isolated from the peripheral blood of acutely relapsing MS patients co-express IFNγ and IL-17 following activation in the presence of IL-23 [48]. Hence, IL-23 might drive the development of ex-T_H17 cells during MS as well as EAE.

Whereas T_H17 cells are highly unstable, the stability of GM-CSF expression by pathogenic T cells is unknown. In mice, T_H1 and T_H17 polarized cells acquire GM-CSF expression upon CNS invasion [36••]. Apparently *in vivo*, the T_H17 transcription factor RORγt is involved in GMCSF expression by T_H cells [35••, 36••], whereas *in vitro*, GM-CSF secretion can be governed by different cytokine receptors and transcription factors [35••]. The long-term commitment of the GM-CSF-secreting pathogenic phenotype and the molecular factors that drive their emergence remain to be investigated in detail.

TH17 cells in MS

The potential importance of T_H17 cells in multiple sclerosis was heralded by early reports of IL-17 gene expression in peripheral blood (PB) and cerebrospinal fluid (CSF) mononuclear cells obtained from MS patients, as well as in MS plaques themselves [49, 50]. The latter observation was made as the result of a microarray analysis comparing fresh-frozen brain tissues obtained at autopsy from MS patients versus controls without CNS pathology [50]. Genes involved in IL-1 and IL-6 signaling, which drive human T_H17 differentiation, were also differentially expressed in the MS lesions [50]. Subsequent studies have used flow cytometry and ELIspot to confirm the presence of T cells producing IL-17A protein at elevated frequencies in the blood and cerebrospinal fluid of untreated individuals with relapsing remitting MS [51–53]. In several of those studies, IL-17A expression was highest during a clinical relapse. Furthermore, two independent groups have documented the accumulation of CD4$^+$ and CD8$^+$ T cells expressing IL-17A protein in active MS lesions by immunohistochemical analysis [48, 54]. One study suggests that the increased frequency of T_H17 cells in MS is secondary to elevated IL-23 production by monocyte derived dendritic cells[55].

Although it remains to be determined whether IL-17 and/ or IL-17 producing T cells directly mediate CNS tissue damage in MS, the administration of disease modifying agents has been associated with a reduction in peripheral T_H17 responses that parallels clinical improvement. This was most recently shown to be the case for fingolimod (FTY-720), the first oral drug to be approved by the Food and Drug Administration to suppress relapses and delay disability progression in patients with relapsing forms of MS [56]. Compared to untreated MS patients or healthy volunteers, MS patients treated with fingolimod had a lower frequency of circulating CD3$^+$ T_H17 cells, as defined by production of IL-17A in response to stimulation with plate-bound anti-CD3 and anti-CD28 *ex vivo* [57]. Similarly, CD4$^+$ T cells staining positive for intracellular IL-17A were reduced in the peripheral blood of MS patients following 6–12 months of treatment with recombinant IFNγ, commonly prescribed as a first line disease modifying agent in relapsing disease [52]. Several mechanisms of action of IFNβ in curtailing T_H17 cells have been proposed. Durelli and colleagues reported that

IFNβ enhances activation-induced apoptosis of T_H17, but not of T_H1, cells [52]. T_H17 cells expressed higher levels of the Type I interferon receptor 1 chain than T_H1 cells, possibly accounting for their increased sensitivity to the cytokine. In an independent study, IFNβ prevented the upregulation of RORc and IL-23 receptor by human $CD4^+CD45RA^+$ lymphocytes cultured under T_H17 polarization conditions. By contrast, IFNβ had no effect on expression of the T_H1 and T_H2 associated transcription factors, T-bet and GATA-3 [58]. IFNβ could also block T_H17 differentiation by suppressing IL-23 and IL-1β and inducing IL-27 secretion by dendritic cells [58, 59]. Collectively, these data are at odds with the findings by Axtell *et al.* that IFNβ therapy suppresses T_H1-mediated, but exacerbates T_H17 mediated, EAE and that an elevated level of IL-17F in the serum of MS patients is predictive of non-responsiveness to IFNβ [60]. Hence the ultimate usefulness of T_H17-related factors as predictive or surrogate biomarkers in MS remains to be established.

It is also unclear why neutralizing antibodies specific for the IL-12/IL-23 p40 chain were ineffective in preventing inflammatory lesion formation or clinical exacerbations in relapsing remitting MS [61, 62]. One possible explanation is that, because of the blood-brain-barrier, anti-IL-12 p40 antibodies did not gain access to the CNS at high enough levels to achieve a notable therapeutic effect. Alternatively, IL-23 might be more important for the initial generation or expansion of autoreactive T cell clones at disease initiation, than for their long-term survival and maintenance in established MS [61]. These issues will only be addressed by future clinical trials of agents that anatagonize T_H17-related factors and have high CNS penetrance.

Concluding remarks

Investigations into the mechanism of action and long-term stability of T_H17 cells in EAE, and of their biological significance in MS, have raised more questions than they have answered. The collective data has shown that exposure of autoreactive T cells to IL-23 is a more accurate determinant of pathogenic potential than the secretion of individual T_H17 associated effector cytokines. The IL-23 induced events that imprint an encephalitogenic program are only now being uncovered. In MS, the accumulation of IL-17 producing T cells in the circulation and CNS compartments correlates with disease activity. However, the failure of anti-IL-12/23 neutralizing antibodies to reduce MS relapse rates and to prevent radiological lesion formation belies the causality of that association. Nonetheless, the identification of myelin-reactive IL-23-driven T cells as encephalitogenic mediators represents an important advance in our understanding of the pathogenesis of autoimmune demyelination. Future research on the subject promises to yield novel candidate biomarkers and therapeutic targets in MS.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

1. O'Garra A, Steinman L, Gijbels K. CD4+ T-cell subsets in autoimmunity. Curr Opin Immunol. 1997; 9:872–883. [PubMed: 9492992]
2. Fouser LA, Wright JF, Dunussi-Joannopoulos K, Collins M. Th17 cytokines and their emerging roles in inflammation and autoimmunity. Immunol Rev. 2008; 226:87–102. [PubMed: 19161418]
3. Littman DR, Rudensky AY. Th17 regulatory T cells in mediating and restraining inflammation. Cell. 2010; 140:845–858. [PubMed: 20303875]

4. Ruuls SR, Sedgwick JD. Cytokine-directed therapies in multiple sclerosis and experimental autoimmune encephalomyelitis. Immunol Cell Biol. 1998; 76:65–73. [PubMed: 9553778]

5. Willenborg DO, Fordham S, Bernard CC, Cowden WB, Ramshaw IA. IFN-gamma plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. J Immunol. 1996; 157:3223–3227. [PubMed: 8871615]

6. Lees JR, Golumbek PT, Sim J, Dorsey D, Russell JH. Regional CNS responses to IFN-gamma determine lesion localization patterns during EAE pathogenesis. J Exp Med. 2008; 205:2633–2642. [PubMed: 18852291]

7. Gutcher I, Urich E, Wolter K, Prinz M, Becher B. Interleukin 18-independent engagement of interleukin 18 receptor-alpha is required for autoimmune inflammation. Nat Immunol. 2006; 7:946–953. [PubMed: 16906165]

8. Liblau R, Steinman L, Brocke S. Experimental autoimmune encephalomyelitis in IL-4-deficient mice. Int Immunol. 1997; 9:799–803. [PubMed: 9184926]

9. Segal BM, Dwyer BK, Shevach EM. An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease. J Exp Med. 1998; 187:537–546. [PubMed: 9463404]

10. Becher B, Durell BG, Noelle RJ. Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12. J Clin Invest. 2002; 110:493–497. [PubMed: 12189243] This is the first published report showing that mice deficient in IL-12p35 develop severe EAE, proposing an encephalitogenic function of IL-23.

11. Gran B, Zhang GX, Yu S, Li J, Chen XH, Ventura ES, Kamoun M, Rostami A. IL-12p35-deficient mice are susceptible to experimental autoimmune encephalomyelitis: evidence for redundancy in the IL-12 system in the induction of central nervous system autoimmune demyelination. J Immunol. 2002; 169:7104–7110. [PubMed: 12471147]

12. Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, Vega F, Yu N, Wang J, Singht K, et al. Novel p19 protein engages IL-12p40 to form a cytokine IL-23, with biological activities similar as well as distinct from IL-12. Immunity. 2000; 13:715–725. [PubMed: 11114383]

13. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, Lucian L, To W, Kwan S, Churakova T, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature. 2003; 421:744–748. [PubMed: 12610626] This paper shows that IL-23(p19)-deficient mice do not develop EAE establishing a non-redundant role of IL-23 in EAE.

14. Gutcher I, Becher B. APC-derived cytokines and T cell polarization in autoimmune inflammation. J Clin Invest. 2007; 117:1119–1127. [PubMed: 17476341]

15. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med. 2005; 201:233–240. [PubMed: 15657292]

16. Kroenke MA, Carlson TJ, Andjelkovic AV, Segal BM. IL-12- IL-23-modulated T cells induce distinct types of EAE based on histology CNS chemokine profile, and response to cytokine inhibition. J Exp Med. 2008 This paper showed that IL-12 and IL-23 polarized myelin-specific T cells could induce clinically identical forms of CNS autoimmune disease by invoking parallel molecular and cellular pathways.

17. Kroenke MA, Segal BM. IL-23 modulated myelin-specific T cells induce EAE via an IFNgamma driven IL-17 independent pathway. Brain Behav Immun. 2011; 25:932–937. [PubMed: 20951792]

18. Komiyama Y, Nakae S, Matsuki T, Nambu A, Ishigame H, Kakuta S, Sudo K, Iwakura Y. IL-17: Plays an important role in the development of experimental autoimmune encephalomyelitis. J Immunol. 2006; 177:566–573. [PubMed: 16785554] This is the first report using IL-17A$^{-/-}$ mice showing that IL-17 production contributes, but is not an absolute requirement for the development of EAE.

19. Hofstetter HH, Ibrahim SM, Koczan D, Kruse N, Weishaupt A, Toyka KV, Gold R. Therapeutic efficacy of IL-17 neutralization in murine experimental autoimmune encephalomyelitis. Cell Immunol. 2005; 237:123–130. [PubMed: 16386239]

20. Haak S, Croxford AL, Kreymborg K, Heppner FL, Pouly S, Becher B, Waisman A. IL-17A andIL-17F do not contribute vitally to autoimmune neuro-inflammation in mice. J Clin Invest. 2009; 119:61–69. [PubMed: 19075395]

21. Kreymborg K, Etzensperger R, Dumoutier L, Haak S, Rebollo A, Buch T, Heppner FL, Renauld JC, Becher B. IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. J Immunol. 2007; 179:8098–8104. [PubMed: 18056351]
22. Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, Schluns K, Tian Q, Watowich SS, Jetten AM, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature. 2007; 448:480–483. [PubMed: 17581589]
23. Coquet JM, Chakravarti S, Smyth MJ, Godfrey DI. Cutting edge: IL-21 is not essential for Th17 differentiation or experimental autoimmune encephalomyelitis. J Immunol. 2008; 180:7097–7101. [PubMed: 18490706]
24. Sonderegger I, Kisielow J, Meier R, King C, Kopf M. IL-21 and IL-21R are not required for development of Th17 cells and autoimmunity in vivo. Eur J Immunol. 2008; 38:1833–1838. [PubMed: 18546146]
25. Steinman L. A rush to judgment on Th17. J Exp Med. 2008; 205:1517–1522. [PubMed: 18591407]
26. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, Giuliani F, Arbour N, Becher B, Prat A. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nat Med. 2007; 13:1173–1175. [PubMed: 17828272] The authors show that IL-17 can alter the integrity and resistance of an *in vitro* model of a human blood–brain barrier and that IL-17/IFN-gamma double positive T cells accumulate in MS lesions.
27. Carlson T, Kroenke M, Rao P, Lane TE, Segal B. The Th17-ELR+ CXC chemokine pathway is essential for the development of central nervous system autoimmune disease. J Exp Med. 2008; 205:811–823. [PubMed: 18347102]
28. Kroenke MA, Chensue SW, Segal BM. EAE mediated by a non-IFN-gamma/non-IL-17 pathway. Eur J Immunol. 2010; 40:2340–2348. [PubMed: 20540117] The authors here demonstrate that in the absence of IL-17 or IFNγ, GM-CSF can act as a potent encephalitogenic factor.
29. Stromnes IM, Cerretti LM, Liggitt D, Harris RA, Goverman JM. Differential regulation of central nervous system autoimmunity by T(H)1 and T(H)17 cells. Nat Med. 2008; 14:337–342. [PubMed: 18278054]
30. McGeachy MJ, Cua DJ. Th17 cell differentiation: the long and winding road. Immunity. 2008; 28:445–453. [PubMed: 18400187]
31. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, Cua DJ. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. Nat Immunol. 2007; 8:1390–1397. [PubMed: 17994024] The authors show that T_H17 cells, generated *in vitro* the presence of IL-6 and TGFβ are incapable of inducing EAE, whereas IL-23-stimulated TH cells are highly pathogenic.
32. Gyulveszi G, Haak S, Becher B. IL-23-driven encephalo-tropism and Th17 polarization during CNS-inflammation in vivo. Eur J Immunol. 2009; 39:1864–1869. [PubMed: 19544494]
33. Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S, Uccelli A, Lanzavecchia A, Engelhardt B, Sallusto F. C-C chemokine receptor 6-regulated entry of T(H)-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. Nat Immunol. 2009; 10:514–523. [PubMed: 19305396]
34. O'Connor RA, Prendergast CT, Sabatos CA, Lau CW, Leech MD, Wraith DC, Anderton SM. Cutting edge: Th1 cells facilitate the entry of Th17 cells to the central nervous system during experimental autoimmune encephalomyelitis. J Immunol. 2008; 181:3750–3754. [PubMed: 18768826]
35. El-Behi M, Ciric B, Dai H, Yan Y, Cullimore M, Safavi F, Zhang GX, Dittel BN, Rostami A. The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. Nat Immunol. 2011; 12:568–575. [PubMed: 21516111] See Ref. [36••].
36. Codarri L, Gyulveszi G, Tosevski V, Hesske L, Fontana A, Magnenat L, Suter T, Becher B. RORgammat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. Nat Immunol. 2011; 12:560–567. [PubMed: 21516112] The authors show that GM-CSF produced by autoreactive TH cells is critical for the induction of IL-23-driven EAE. They show that *in vivo*, the T_H17 transcription factor Rorγt is involved in the expression of GM-CSF. T cells lacking IL-17 and IFNγ, when expressing GM-

CSF are fully pathogenic, whereas T cells expressing IFNγ and IL-17 but lacking GM-CSF cannot induce CNS inflammation.

37. McQualter JL, Darwiche R, Ewing C, Onuki M, Kay TW, Hamilton JA, Reid HH, Bernard CC. Granulocyte macrophage colony-stimulating factor: a new putative therapeutic target in multiple sclerosis. J Exp Med. 2001; 194:873–882. [PubMed: 11581310]
38. King IL, Dickendesher TL, Segal BM. Circulating Ly-6C+ myeloid precursors migrate to the CNS and play a pathogenic role during autoimmune demyelinating disease. Blood. 2009; 113:3190–3197. [PubMed: 19196868] This paper provides a mechanism of action of GM-CSF in EAE by demonstrating its role in the mobilization of Ly6C+ monocytes that infiltrate the CNS and differentiate into dendritic cells.
39. Deshpande P, King IL, Segal BM. Cutting edge: CNS CD11c+ cells from mice with encephalomyelitis polarize Th17 cells and support CD25+CD4+ T cell-mediated immunosuppression, suggesting dual roles in the disease process. J Immunol. 2007; 178:6695–6699. [PubMed: 17513712]
40. Greter M, Heppner FL, Lemos MP, Odermatt BM, Goebels N, Laufer T, Noelle RJ, Becher B. Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. Nat Med. 2005; 11:328–334. [PubMed: 15735653]
41. McMahon EJ, Bailey SL, Castenada CV, Waldner H, Miller SD. Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. Nat Med. 2005; 11:335–339. [PubMed: 15735651]
42. King IL, Kroenke MA, Segal BM. GM-CSF-dependent, CD103+ dermal dendritic cells play a critical role in Th effector cell differentiation after subcutaneous immunization. J Exp Med. 2010; 207:953–961. [PubMed: 20421390]
43. Lee YK, Turner H, Maynard CL, Oliver JR, Chen D, Elson CO, Weaver CT. Late developmental plasticity in the T helper 17 lineage. Immunity. 2008; 30:92–107. [PubMed: 19119024] This pioneering study demonstrated the plasticity of human Th17 cells and their ability to acquire the characteristics of Th1 cells.
44. Annunziato F, Romagnani S. The transient nature of the Th17 phenotype. Eur J Immunol. 2010; 40:3312–3316. [PubMed: 21110314]
45. Hirota K, Duarte JH, Veldhoen M, Hornsby E, Li Y, Cua DJ, Ahlfors H, Wilhelm C, Tolaini M, Menzel U, et al. Fate mapping of IL-17-producing T cells in inflammatory responses. Nat Immunol. 2011; 12:255–263. [PubMed: 21278737] See Ref. [47•].
46. Bending D, De la Pena H, Veldhoen M, Phillips JM, Uyttenhove C, Stockinger B, Cooke A. Highly purified Th17 cells from BDC2. 5NOD mice convert into Th1-like cells in NOD/SCID recipient mice. J Clin Invest. 2009; 119:565–572. [PubMed: 19188681]
47. Kurschus FC, Croxford AL, Heinen AP, Wortge S, Ielo D, Waisman A. Genetic proof for the transient nature of the Th17 phenotype. Eur J Immunol. 2010; 40:3336–3346. [PubMed: 21110317] These studies show that a significant percentage of T cells infiltrating the CNS during EAE are ‘ex-Th17’ cells (TH17 cells that have upregulated IFNγ and T-bet and downregulated IL-17).
48. Kebir H, Ifergan I, Alvarez JI, Bernard M, Poirier J, Arbour N, Duquette P, Prat A. Preferential recruitment of interferon-gamma-expressing TH17 cells in multiple sclerosis. Ann Neurol. 2009; 66:390–402. [PubMed: 19810097]
49. Matusevicius D, Kivisakk P, He B, Kostulas N, Ozenci V, Fredrikson S, Link H. Interleukin-17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis. Mult Scler. 1999; 5:101–104. [PubMed: 10335518]
50. Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, Langer-Gould A, Strober S, Cannella B, Allard J, et al. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med. 2002; 8:500–508. [PubMed: 11984595]
51. Brucklacher-Waldert V, Stuerner K, Kolster M, Wolthausen J, Tolosa E. Phenotypical and functional characterization of T helper 17 cells in multiple sclerosis. Brain. 2009; 132:3329–3341. [PubMed: 19933767]

52. Durelli L, Conti L, Clerico M, Boselli D, Contessa G, Ripellino P, Ferrero B, Eid P, Novelli F. T-helper 17 cells expand in multiple sclerosis and are inhibited by interferon-beta. Ann Neurol. 2009; 65:499–509. [PubMed: 19475668]

53. Li Y, Wang H, Long Y, Lu Z, Hu X. Increased memory Th17 cells in patients with neuromyelitis optica and multiple sclerosis. J Neuroimmunol. 2011; 234:155–160. [PubMed: 21489641]

54. Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM, Fugger L. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. Am J Pathol. 2008; 172:146–155. [PubMed: 18156204]

55. Vaknin-Dembinsky A, Balashov K, Weiner HL. IL-23 is increased in dendritic cells in multiple sclerosis and down-regulation of IL-23 by antisense oligos increases dendritic cell IL-10 production. J Immunol. 2006; 176:7768–7774. [PubMed: 16751425]

56. Cohen JA, Chun J. Mechanisms of fingolimod's efficacy and adverse effects in multiple sclerosis. Ann Neurol. 2011; 69:759–777. [PubMed: 21520239]

57. Mehling M, Lindberg R, Raulf F, Kuhle J, Hess C, Kappos L, Brinkmann V. Th17 central memory T cells are reduced by FTY720 in patients with multiple sclerosis. Neurology. 2010; 75:403–410. [PubMed: 20592255]

58. Ramgolam VS, Sha Y, Jin J, Zhang X, Markovic-Plese S. IFN-beta inhibits human Th17 cell differentiation. J Immunol. 2009; 183:5418–5427. [PubMed: 19783688]

59. Sweeney CM, Lonergan R, Basdeo SA, Kinsella K, Dungan LS, Higgins SC, Kelly PJ, Costelloe L, Tubridy N, Mills KH et al. IL-27 mediates the response to IFN-beta therapy in multiple sclerosis patients by inhibiting Th17 cells. Brain Behav Immun. 2011; 25:1170–1181. [PubMed: 21420486]

60. Axtell RC, de Jong BA, Boniface K, van der Voort LF, Bhat R, De Sarno P, Naves R, Han M, Zhong F, Castellanos JG, et al. T helper type 1 and 17 cells determine efficacy of interferon-beta in multiple sclerosis and experimental encephalomyelitis. Nat Med. 2010; 16:406–412. [PubMed: 20348925]

61. Segal BM, Constantinescu CS, Raychaudhuri A, Kim L, Fidelus-Gort R, Kasper LH. Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study. Lancet Neurol. 2008; 7:796–804. [PubMed: 18703004]

62. Vollmer TL, Wynn DR, Alam MS, Valdes J. A phase 2, 24-week, randomized, placebo-controlled, double-blind study examining the efficacy and safety of an anti-interleukin-12 and-23 monoclonal antibody in patients with relapsing-remitting or secondary progressive multiple sclerosis. Mult Scler. 2011; 17:181–191. [PubMed: 21135022]