Commentary

Interleukin-17—Extended Features of a Key Player in Multiple Sclerosis

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Although multiple sclerosis (MS) is the most common neurological disease of young adults, afflicting hundreds of thousands of people worldwide, its pathogenesis is still only incompletely understood. There seems to be substantial heterogeneity in disease mechanisms, but in the majority of cases an autoimmune origin or at least a decisive autoimmune component is postulated. Therefore, pathogenetic research focuses on different players programmed by the immune system. In this issue of *The American Journal of Pathology* Tzartos and colleagues¹ extend previous findings about the role of the effector cytokine interleukin (IL)-17 in human disease. IL-17 has recently joined the club of molecules considered as important immunological players for inflammation in the nervous system.

In the past a multitude of studies has addressed the role of the so-called type 1 and type 2 cytokines for adaptive immune responses in MS and its corresponding animal model, experimental autoimmune encephalomyelitis (EAE). The general notion has included that a type 1 response (with CD4 T cells of helper type 1, TH1 cells) represents a proinflammatory, destructive immune reaction whereas a type 2 response (with TH2 cells) reflects a modulatory, nonpathogenic immune reaction and can even protect from autoimmune disease caused by TH1-dependent mechanisms.² Study in experimental systems has suggested that TH1 and TH2 cells represent terminally differentiated lineages. Critical effector cytokines in a TH1 response are interferon- γ and tumor necrosis factor- α , which are both implicated in mediating disease pathology in MS and EAE. T-bet, STAT4, and STAT1 are TH1-associated transcription factors. Similarly a TH2 response is characterized by cytokine secretion of IL-4, IL-5, and IL-13 and the transcription factor STAT6.

Because MS and especially EAE were originally considered as TH1-mediated autoimmune diseases, IL-12 secreted by antigen-presenting cells has commonly been implicated as the critical upstream cytokine for the underlying TH1 response, the presence or absence of

which determines the character of an evolving immune reaction.³ This view was later revised when it was realized that indeed IL-12 knockout mice (which lack the specific p35 subunit and not the p40 subunit shared by IL-12 and IL-23) are highly susceptible to EAE induction whereas IL-23 was discovered to be the upstream modulator of the pathogenic signaling pathways.⁴ Because IL-23 is crucially involved in the expansion of cells producing IL-17, this view converged with the discovery that IL-17 is produced by TH cells that are distinct from the traditional TH1 and TH2 cell subsets and were thus designated as TH17 cells.⁵ Requirements for TH17 cell differentiation were first defined in experimental models (Figure 1). Here, it was shown in parallel by several groups that a combination of IL-6 and transforming growth factor- β is required and that differentiated TH17 cells are maintained and expanded by IL-23, which is unable to drive TH17 differentiation of naïve T cells by itself.^{6,7} Besides IL-17A and IL-17F, these cells produce IL-6, IL-21, IL-22, tumor necrosis factor- α , and granulocyte macrophage-colony stimulating factor.^{8,9} At the molecular level the transcription factor ROR γ T (or the human RORC variant 2 ortholog) serves as the master switch.¹⁰ Additionally, STAT3, which is activated by IL-6 and IL-21, acts in concert with ROR γ T.¹¹ Very recent data show that the interferon regulatory factor-4 also seems to be critically involved, because *Irfl*-deficient mice fail to produce IL-17.¹² In contrast, the transcriptional master switch for TH1 cells, T-bet, seems to antagonize TH17 differentiation as does STAT1. The TH1- and TH2-associated STAT4 and STAT6, respectively, are not involved in TH17 differentiation.¹² In the presence of transforming growth factor- β without IL-6 stimulation, there occurs differentiation to regulatory T cells, and it could be convincingly shown that this population originates from the same precursor cells.⁷ These cells are able to regulate TH17 cells, as they do for TH1 and TH2 cells, pointing to a reciprocal

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Figure 1. Molecules involved in the differentiation of murine TH17 cells. Several positive and negative feedback loops are involved, indicated by + and -, respectively. **Dashed arrows** indicate pathways with limited evidence. See text for details and abbreviations.

development and a functional dichotomy between these CD4 T-cell subtypes, depending on the state of the innate immune system, the cytokine microenvironment, and therefore the functional needs. Thus, this rapidly emerging set of data clearly demonstrates that TH17 cells are indeed a T-helper cell subpopulation distinct in differentiation and function from the TH1 and TH2 subsets described previously.

IL-17 itself is a crucial effector cytokine with potent proinflammatory effects. It induces the expression of other proinflammatory cytokines such as tumor necrosis factor- α and chemokines, attracts neutrophilic leukocytes, and enhances the maturation of dendritic cells.¹³ In the context of acquired immunity, IL-17-producing cells synergize for the control of a variety of bacterial and fungal infections at mucosal surfaces, and IL-17 and IL-22 cooperatively enhance the expression of antimicrobial peptides.⁸ Beyond this invaluable contribution to host defense, IL-17-producing cells are thought to be essential inflammatory mediators in autoimmune diseases such as collagen-induced arthritis, colitis, psoriasis, and EAE. We and others have previously observed that TH17 cells in EAE are CD4 cells and that they are present both in the immune periphery and in the inflamed central nervous system in EAE induced by the MOG-peptide 35-55 in C57.BL/6 mice¹⁴ and in PLP-induced EAE in SJL mice.¹⁵ Moreover, neutralization of IL-17 ameliorates clinical disease,¹⁶ a finding that is paralleled by reduced EAE severity in IL-17-deficient animals.¹⁷ It could also be demonstrated that immature TH17 cells exist in the thymus of mice.¹⁸ An interesting new observation is that pertussis toxin, which is widely used as adjuvant for EAE induction in combination with PLP and incomplete Freund's adjuvant, is able to induce PLP-specific IL-17-producing CD4 T cells in the periphery.¹⁹ Therefore, stimulation of the innate immune system with unrelated environmental pathogens can drive the adaptive immune system toward TH17 differentiation, which in turn can support organ-specific autoimmunity provided that the organism is genetically prone for the development of autoimmunity.

All these experimental analyses concerning IL-17 in mice and EAE focused on CD4 T cells, whereas little is

yet known about CD8 T cells. The report in this issue of the *AJP* by Tzartos and colleagues¹ adds some new interesting observations concerning the human disease. A systematic analysis of IL-17-positive cells in the brains of MS patients revealed a significant increase in the number of IL-17⁺ T cells in the active rather than the inactive areas of MS lesions. Importantly, CD8 T cells were positively immunostained for IL-17 at frequencies similar to those of CD4 T cells in MS tissues. In recent years our view of effector-cell populations in MS has been revised. Whereas EAE models with their intrinsic bias toward CD4 T cells²⁰ have influenced our previous therapeutic strategies, the failure of CD4-directed therapies in MS²¹ and the clonal expansion of CD8 T cells in MS lesions and cerebrospinal fluid²² may identify the true bad guy in the inflamed brain. CD8 T cells came into the focus of MS research when it was demonstrated that CD8⁺ T cells are more frequent in chronically inflamed MS plaques compared with CD4⁺ T cells and that neurons are able to up-regulate MHC I molecules after exposure to interferon- γ , which renders them vulnerable to cytotoxic T-cell attack.²³ Furthermore, axonal damage in MS lesions correlates with the number of CD8 T cells but not with TH subsets. The linkage between CD8 T cells and IL-17 as a crucial effector cytokine, which is described here, adds an important piece of data for future approaches in MS. Also, these findings corroborate previous microarray analyses of MS lesions obtained at autopsy that demonstrated highly increased transcripts of IL-17.²⁴ Unfortunately, there exist only very few CD8 T-cell-based EAE models at the moment. It would be of great interest to further develop such models to elucidate the contribution of CD8 T cells also in light of the potential role of IL-17 as an effector cytokine in cytotoxic T cells.

Another exciting finding was the fact that IL-17 immunoreactivity could also be detected in astrocytes and oligodendrocytes in active areas of MS plaques. Previously, IL-17 had been found to be expressed in human astrocyte cultures and shown to be up-regulated by tumor necrosis factor- α and IL-1 β .²⁵ Additionally, IL-17-positive astrocytes were found in human and rat brains with acute cerebral ischemia. Therefore, it seems likely that astrocytes (and perhaps oligodendrocytes) can be forced to produce IL-17 under the influence of activating stimuli such as ischemic stress or inflammatory cytokines. This can well be one source, besides infiltrating TH17 cells, for the elevated IL-17 levels found in the cerebrospinal fluid of patients with opticospinal MS.²⁶ The biological role of this extra T-cell IL-17 remains unclear at the moment, but one can envision a proinflammatory amplification loop resulting in the brain. Future investigations should aim at identifying how such intense inflammation can be terminated, eg, by negative feedback loops.

Because it becomes increasingly clear that IL-17 is an essential player in MS, possibilities for therapeutic intervention are warranted. This, however, does not seem to be an easy task. In the absence of IL-6, FoxP3-positive regulatory T cells are educated,⁷ which can modulate inflammation. Indeed, a functional deficit but not a reduction in absolute numbers of regulatory T cells was demonstrated in MS

patients.²⁷ However, when IL-6 is lacking, as can be achieved in knockout mice, IL-21 can step in and initiate an alternative pathway to induce proinflammatory TH17 cells, as recently shown.²⁸ Indeed, IL-6 seems only to be the first cytokine within a cascade compromising IL-21, which acts in an autocrine amplification loop, and later IL-23, which drives TH17 differentiation (Figure 1). That IL-23 cannot drive TH17 cell differentiation on its own is probably only attributable to the fact that IL-23R is not expressed on naïve T cells. Thus, one can speculate that any signal that up-regulates IL-23R may promote TH17 cell differentiation in an appropriate environment. Therefore, the TH17 cell differentiation pathways get complex, and a potential therapy has to choose carefully an appropriate target. Moreover, there are unexpected species differences, with IL-1 β instead of transforming growth factor- β reported to be crucial for TH17 cell differentiation in humans,²⁹ making it more difficult to find adequate therapeutic approaches in animal models. Another unsolved question is the potential interconvertibility of TH17 cells and whether there is flexibility in cytokine production, which can be used therapeutically.

More than 2 decades after coining of the TH1/TH2 paradigm, new players have entered the field of autoimmunity. We have learned from previous trials in MS that cytokine networks are often redundant and great care is required when experimental findings from rodent species are translated into human therapy. The findings by Tzartos and colleagues¹ are an important step on this challenging road. They will not only stimulate cytokine-directed therapy but also require specific care when experimental paradigms are transferred to patients.

References

1. Tzartos JS, Friese MA, Craner M, Palace J, Newcombe J, Esiri MM, Fugger L: Interleukin-17 production in CNS-infiltrating T-cells and glial cells is associated with active disease in multiple sclerosis. *Am J Pathol* 2007, 172:146-155
2. Röcken M, Shevach EM: Immune deviation—the third dimension of nondeletional T cell tolerance. *Immunol Rev* 1996, 149:175-194
3. Gately MK, Renzetti LM, Magram J, Stern AS, Adorini L, Gubler U, Presky DH: The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol* 1998, 16:495-521
4. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, Lucian L, To W, Kwan S, Churakova T, Zurawski S, Wiekowski M, Lira SA, Gorman D, Kastelein RA, Sedgwick JD: Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 2003, 421:744-748
5. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT: Interleukin 17-producing CD4(+) effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005, 6:1123-1132
6. Stockinger B, Veldhoen M: Differentiation and function of Th17 T cells. *Curr Opin Immunol* 2007, 19:281-286
7. Bettelli E, Carrier YJ, Gao WD, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK: Reciprocal developmental pathways for the generation of pathogenic effector T(H)17 and regulatory T cells. *Nature* 2006, 441:235-238
8. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA: Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 2006, 203:2271-2279
9. Bettelli E, Oukka M, Kuchroo VK: T-H-17 cells in the circle of immunity and autoimmunity. *Nat Immunol* 2007, 8:345-350
10. Ivanov IL, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR: The orphan nuclear receptor ROR γ directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 2006, 126:1121-1133
11. Zhou L, Ivanov IL, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR: IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* 2007, 8:967-974
12. Brüstle A, Heink S, Huber M, Rosenplanter C, Stadelmann C, Yu P, Arpaia E, Mak TW, Kamradt T, Lohoff M: The development of inflammatory T(H)-17 cells requires interferon-regulatory factor 4. *Nat Immunol* 2007, 8:958-966
13. Kolls JK, Linden A: Interleukin-17 family members and inflammation. *Immunity* 2004, 21:467-476
14. 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
15. Hofstetter HH, Toyka KV, Tary-Lehmann M, Lehmann PV: Kinetics and organ distribution of IL-17-producing CD4 cells in proteolipid protein 139-151 peptide-induced experimental autoimmune encephalomyelitis of SJL mice. *J Immunol* 2007, 178:1372-1378
16. 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
17. 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
18. Hofstetter HH, Lühder F, Toyka KV, Gold R: IL-17 production by thymocytes upon CD3 stimulation and costimulation with microbial factors. *Cytokine* 2006, 34:184-197
19. Hofstetter HH, Grau C, Buttmann M, Forsthuber TG, Gaupp S, Toyka KV, Gold R: The PLPP-specific T cell population promoted by Pertussis toxin is characterized by high frequencies of IL-17 producing cells. *Cytokine* 40:35-43
20. Gold R, Linington C, Lassmann H: Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. *Brain* 2006, 129:1953-1971
21. Hohlfeld R, Wekerle H: Drug insight: using monoclonal antibodies to treat multiple sclerosis. *Nat Clin Pract Neurol* 2005, 1:34-44
22. Skulina C, Schmidt S, Dornmair K, Babbe H, Roers A, Rajewsky K, Wekerle H, Hohlfeld R, Goebels N: Multiple sclerosis: brain-infiltrating CD8(+) T cells persist as clonal expansions in the cerebrospinal fluid and blood. *Proc Natl Acad Sci USA* 101:2428-2433
23. Friese MA, Fugger L: Autoreactive CD8(+) T cells in multiple sclerosis: a new target for therapy? *Brain* 2005, 128:1747-1763
24. Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, Langer-Gould A, Strober S, Cannella B, Allard J, Klonowski P, Austin A, Lad N, Kaminski N, Galli SJ, Oksenberg JR, Raine CS, Heller R, Steinman L: Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat Med* 2002, 8:500-508
25. Meeuwssen S, Persoon-Deen C, Bsibsi M, Ravid R, van Noort JM: Cytokine, chemokine and growth factor gene profiling of cultured human astrocytes after exposure to proinflammatory stimuli. *Glia* 2003, 43:243-253
26. Ishizu T, Osoegawa M, Mei FJ, Kikuchi H, Tanaka M, Takakura Y, Minohara M, Murai H, Mihara F, Taniwaki T, Kira J: Intrathecal activation of the IL-17/IL-8 axis in opticospinal multiple sclerosis. *Brain* 2005, 128:988-1002
27. Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA: Loss of functional suppression by CD4(+)CD25(+) regulatory T cells in patients with multiple sclerosis. *J Exp Med* 2004, 199:971-979
28. Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, Oukka M, Kuchroo VK: IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* 2007, 448:484-487
29. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F: Interleukins 1 β and 6 but not transforming growth factor- β are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* 2007, 8:942-949