IL-17 Plays an Important Role in the Development of Experimental Autoimmune Encephalomyelitis

Yutaka Komiyama, Susumu Nakae, Taizo Matsuki, Aya Nambu, Harumichi Ishigame, Shigeru Kakuta, Katsuko Sudo and Yoichiro Iwakura

*J Immunol* 2006; 177:566-573; doi: 10.4049/jimmunol.177.1.566
http://www.jimmunol.org/content/177/1/566

References
This article cites 50 articles, 31 of which you can access for free at:
http://www.jimmunol.org/content/177/1/566.full#ref-list-1

Subscriptions
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscriptions

Permissions
Submit copyright permission requests at:
http://www.aai.org/ji/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/cgi/alerts/etoc
IL-17 Plays an Important Role in the Development of Experimental Autoimmune Encephalomyelitis

Yutaka Komiyama, Susumu Nakae, Taizo Matsuki, Aya Nambu, Harumichi Ishigame, Shigeru Kakuta, Katsuko Sudo, and Yoichiro Iwakura

IL-17 is a proinflammatory cytokine that activates T cells and other immune cells to produce a variety of cytokines, chemokines, and cell adhesion molecules. This cytokine is augmented in the sera and/or tissues of patients with contact dermatitis, asthma, and rheumatoid arthritis. We previously demonstrated that IL-17 is involved in the development of autoimmune arthritis and contact, delayed, and airway hypersensitivity in mice. As the expression of IL-17 is also augmented in multiple sclerosis, we examined the involvement of this cytokine in these diseases using IL-17−/− murine disease models. We found that the development of experimental autoimmune encephalomyelitis (EAE), the rodent model of multiple sclerosis, was significantly suppressed in IL-17−/− mice; these animals exhibited delayed onset, reduced maximum severity scores, ameliorated histological changes, and early recovery. T cell sensitization against myelin oligodendrocyte glycoprotein was reduced in IL-17−/− mice upon sensitization. The major producer of IL-17 upon treatment with myelin digodendrocyte glycoprotein was CD4+ T cells, and adoptive transfer of IL-17−/− CD4+ T cells inefficiently induced EAE in recipient mice. Notably, IL-17-producing T cells were increased in IFN-γ−/− cells, while IFN-γ-producing cells were increased in IL-17−/− cells, suggesting that IL-17 and IFN-γ mutually regulate IFN-γ and IL-17 production. These observations indicate that IL-17 rather than IFN-γ plays a crucial role in the development of EAE. The Journal of Immunology, 2006, 177: 566–573.

Copyright © 2006 by The American Association of Immunologists, Inc. 0022-1767/06/$02.00

Received for publication August 30, 2005. Accepted for publication April 7, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by grants from the Ministry of Education, Culture, Sports, and Science of Japan, and the Ministry of Health and Welfare of Japan.

2 Current address: Department of Pathology, Stanford University School of Medicine, 269 Campus Drive, Center for Clinical Sciences Research 3255, Stanford, CA 94305-5176.

3 Current address: ERATO Yanagisawa Orphan Receptor Project, Japan Science and Technology Agency, Koto-Ku, Japan.

4 Current address: Animal Research Center, Tokyo Medical University, Sinjyuku-ku, Tokyo 160-8402, Japan.

5 Address correspondence and reprint requests to Dr. Yoichiro Iwakura, Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. E-mail address: iwakura@ims.u-tokyo.ac.jp

Abbreviations used in this paper: RA, rheumatoid arthritis; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; MOG, myelin oligodendrocyte glycoprotein; PTT, pertussis toxin; LN, lymph node; rm, recombinant human; GVHR, graft-vs-host reaction; CIA, collagen-induced arthritis; IDDM, insulin-dependent diabetes mellitus.
(MOG)-specific T cells. In this model, CD4+ T cells were the major producers of IL-17 in this system.

**Materials and Methods**

**Mice**

IL-17−/− mice (9), generated as described previously, were backcrossed to the C57BL/6J strain (six or nine generations). C57BL/6J and IFN-γ−/− mice, both on the C57BL/6J background were purchased from Japan SLC and from The Jackson Laboratory, respectively. Mice were kept under pathogen-free conditions in an environmentally controlled clean room at the Center for Experimental Medicine, Institute of Medical Science, University of Tokyo. All experiments were conducted according to the institutional ethical guidelines for animal experimentation and the safety guidelines for genetic manipulation experiments.

**Induction of EAE**

**Active EAE.** The MOG35–55 peptide (MEVGWYRSPFSRVVHLYRNGK) was synthesized and purified by HPLC at our institute (Dr. S. Imajoh-Ohmi, Division of Molecular Biology, Institute of Medical Science, University of Tokyo). Mice (8–13 wk of age) were immunized s.c. in one flank on day 0 and in the other on day 7 with 300 μg of MOG35–55 peptide emulsified in CFA (1:1), which consisted of IFA with 5 mg/ml Mycobacterium tuberculosi s H37RA (Difco Laboratories). Pertussis toxin (PTx; Alexis) (200 ng) was injected i.v. on days 0 and 2. Following the first immunization, the severity of EAE was monitored and graded on a scale of 0–5: 0, no disease; 1, limp tail; 2, hind limb weakness; 3, hind limb paralysis; 4, hind and fore limb paralysis; 5, moribundity and death.

**Passive EAE.** Mice were immunized s.c. with MOG/CFA. Ten days after the immunization, the spleen and inguinal and axillary lymph nodes (LNs) were collected and a single-cell suspension was prepared. Pooled lymphocytes (4 × 10^6 cells/ml) were cultured in the presence of 50 μg/ml MOG35–55 peptide for 72 h as described above. To examine IL-17 production by Gr-1+ neutrophils, we prepared a single-cell suspension from the spleens of EAE-affected wild-type mice (day 42). LN cells (72 h after cultivation) and spleen cells were stimulated for 6 h with 20 ng/ml PMA (Sigma-Aldrich), 1 μM ionomycin (Sigma-Aldrich), and 2 μM monensin (Sigma-Aldrich) for 6 h. After harvesting, cells were incubated in ice with anti-mouse CD16/CD32 mAb (2.4G2) in a staining buffer on ice for 15 min. Cell samples were then incubated on ice with either FITC-anti-mouse CD3 or FITC-anti-mouse B220 (RA3-6B2), FITC anti-mouse Gr-1 (RB6-8C5), or FITC anti-mouse CD3ε (145-2C11), allophycocyanin anti-mouse CD4 (RM4-5), 7-Aminoactinomycin D (Sigma-Aldrich)-negative, and 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) (Milenyi Biotec). The sections at the lumbar level are shown.

**Histology**

On day 42 after the first immunization with MOG/CFA and PTx, spines and spinal cords were removed. The tissue sections were stained with H&E. Histological sections were prepared. LN cells were harvested and fixed with neutral 10% formalin. Spinal cords were removed. The tissue sections were stained with H&E.

**Measurement of cytokine levels by ELISA**

To detect IFN-γ and IL-4 in culture supernatants, we used mouse IFN-γ OptEIA kit (BD Pharmingen) and IL-4 ELISA kit (Endogen). Detection of IL-17 by ELISA was performed as described previously (9).

**Flow cytometry**

To examine LN cell population, inguinal and axillary LN cells were harvested 10 days after immunization with MOG/CFA. After incubation of cells on ice with anti-mouse CD16/CD32 mAb (2.4G2) in a staining buffer (Hank’s buffer containing 2% FCS and 0.1% sodium azide) on ice for 15 min, cells were incubated on ice for 45 min with either FITC-anti-mouse CD45RB (H9262) or FITC anti-mouse CD62L (H11001) in the presence of PE-conjugated anti-mouse IL-17 (AbD Biosciences), respectively. Mice were immunized s.c. with MOG/CFA. Ten days after the immunization, the spleen and inguinal and axillary lymph nodes (LNs) were collected and a single-cell suspension was prepared. Pooled lymphocytes (4 × 10^6 cells/ml) were cultured in the presence of 50 μg/ml MOG35–55 peptide for 72 h as described above. To examine IL-17 production by Gr-1+ neutrophils, we prepared a single-cell suspension from the spleens of EAE-affected wild-type mice (day 42). LN cells (72 h after cultivation) and spleen cells were stimulated for 6 h with 20 ng/ml PMA (Sigma-Aldrich), 1 μM ionomycin (Sigma-Aldrich), and 2 μM monensin (Sigma-Aldrich) for 6 h. After harvesting, cells were incubated in ice with anti-mouse CD16/CD32 mAb (2.4G2) in a staining buffer on ice for 15 min. Cell samples were then incubated on ice for 45 min with either FITC-anti-mouse B220 (RA3-6B2), FITC anti-mouse Gr-1 (RB6-8C5), or FITC anti-mouse CD3ε (145-2C11), allophycocyanin anti-mouse CD4 (RM4-5 or GK1.5), or allophycocyanin anti-mouse CD8α (53-6.7) Abs. After washing, the cells were fixed in a fixation buffer (2% paraformaldehyde in PBS) at room temperature for 10 min. Samples were then permeabilized with permeabilization buffer (staining buffer containing 0.1% saponin) and incubated for 30 min with PE-conjugated anti-mouse IL-17 mAb (TC11-18H10) or isotype-matched control rat IgG1 (R3-34) at 4°C. The cells were analyzed on a FACSCalibur flow cytometer as described above. All mAbs were purchased from BD Pharmingen.

**CD4+ T cell cultures**

CD4+ T cells (<95%) from wild-type mouse spleen were purified by MACS system as described above (9), then stimulated with 1.0 μg/ml plate-coated anti-CD3 mAb (145-2C11; BD Pharmingen) in the presence or absence of various concentrations of recombinant mouse IFN-γ.
was significantly delayed in IL-17-/- mice. Initial signs of EAE were observed 10 days after the first immunization, although the severity of disease in IL-17-/- mice was milder than that in wild-type mice (Fig. 1A). Consistent with these observations, a massive infiltration of mononuclear cells was observed within the spinal cords of wild-type mice 42 days after the first immunization (Fig. 2, B and D). In contrast, the cellular infiltration was significantly reduced in IL-17-/- mice (Fig. 2, A and C).

PTx is widely used to enhance the development of T cell-mediated organ-specific autoimmune diseases, including EAE. TNF-α/-/- mice develop EAE normally when high doses of PTx are injected, while in the presence of low doses of PTx, mutant mice exhibit significantly reduced development of EAE symptoms (21). Thus, PTx may sometimes mask or compensate for the pathological functions of some proinflammatory mediators, such as TNF-α, in the pathogenesis of EAE. Therefore, to more clearly observe the effect of IL-17 deficiency, we next induced EAE in IL-17-/- mice in the absence of PTx treatment. Under these conditions, disease onset in IL-17-/- mice delayed compared with wild-type mice similarly to that seen in the presence of PTx (Fig. 3). Interestingly, in the absence of PTx, the disease also gradually ameliorated in wild-type mice after 22 days of induction, as seen for IL-17-/- mice, although the maximal severity score of the wild-type mice remained significantly higher (Fig. 3). These results demonstrate that IL-17 contributes to the development of EAE.

MOG-specific T cell sensitization was impaired in IL-17-/- mice

We previously showed that IL-17 plays an important role in Ag-specific T cell activation during the development of multiple allergic and autoimmune diseases (9–11). To compare our previous

**FIGURE 4.** MOG-specific lymphocyte activation was impaired in IL-17-/- mice. Mice were immunized once with MOG/CFA in the absence of PTx, and 10 days after immunization, the inguinal and axillary LN s were collected and pooled. A, Total LN cell number. B and C, Percentage of CD62L-CD44high or CD45RBlowCD44high CD4+ T cells in LNs. In A and C, each circle represents a value from an individual mouse, and the columns represent the average for each group. Representative FACS results are shown in B. *p < 0.05 and vs the corresponding values for wild-type mice. D, Isolated LN cells were cultured in the absence or presence of MOG peptide for 72 h, and MOG-specific LN cell proliferation was measured by [3H]thymidine incorporation (D) and IFN-γ, IL-17, and IL-4 levels in the culture supernatants (2 x 10^5 cells) (E) are shown. O, Wild-type mice, and ●, IL-17-/- mice. Averages ± SD of triplicate wells are shown. All results are representative at least in three experiments. D, Data are averages ± SD from three independent experiments. N.D., Not detected.
results to the models in the current study, we next assessed the role of IL-17 in the activation of MOG-specific T cells during the development of EAE. Ten days after immunization with MOG/CFA alone (without PTx), hypertrophy of inguinal and axillary lymph nodes was observed. Although the total number of pooled inguinal and axillary LN cells was significantly decreased in IL-17−/− mice in comparison with wild-type mice (Fig. 4A), the content of memory CD4+ T cells (CD62L−CD44high or CD45RBlowCD44high) was comparable (Fig. 4, B and C). Then, the draining LN cells were cultured in the presence or absence of MOG peptide. When a large excess of LN cells (4 × 10^5 cells) was present in a well, the observed MOG-specific LN cell proliferative responses in IL-17−/− mice were similar to those seen in wild-type LN cells (Fig. 4D). In the presence of an optimal number of LN cells (2 × 10^5 and 1 × 10^5 cells), the proliferative responses of cells derived from IL-17−/− mice were markedly decreased compared with those in wild-type mice despite similar number of memory T cells was contained in wild-type and IL-17−/− mouse culture (Fig. 4D). IL-17 was detected in the supernatants of wild-type LN cell cultures (2 × 10^5 cells), and its levels increased in a manner dependent on the dose of MOG peptide. IL-17 was undetectable in IL-17−/− LN cell cultures (2 × 10^5 cells) (Fig. 4D). The MOG-specific proliferative responses of LN T cells were reduced in IL-17−/− mice (Fig. 4D). Nevertheless, IFN-γ production in the LN cell culture supernatants was similar in wild-type and IL-17−/− mice (Fig. 4E). IL-4 levels in the LN cell culture supernatants from both wild-type and IL-17−/− mice were below the limit of detection (Fig. 4E). These results suggest that the delayed onset of the EAE response in IL-17−/− mice is caused by insufficient T cell sensitization against the MOG peptide.

**CD4+ T cells produced IL-17 in LN cells during EAE**

Different subsets of CD4+ Th cells and eosinophils are known to produce IL-17 in patients with dermatitis, RA, Lyme arthritis, and asthma (13–16). Neutrophils and CD8+ T cells can also produce IL-17 during certain infectious diseases in mice (17, 18). These observations suggest that the IL-17 producer cells may differ from those known to produce this cytokine in other diseases. Thus, we next analyzed the IL-17 producer cells in the LNs of wild-type mice following MOG immunization. Ten days after immunization, inguinal and axillary LNs were collected and LN cells were cultured in the presence of MOG peptide for 72 h. After MOG stimulation, IL-17 production was detected in CD3+ T cells, but not in granulocytes or B cells (Fig. 5A). Within the T cell population, IL-17 was predominantly produced in CD4+ T cells, but at low levels in CD8+ T cells (Fig. 5B). Thus, CD4+ T cells, rather than CD8+ T cells, were the major producer of IL-17 within LNs during the development of EAE.

The efficiency of EAE induction of IL-17−/− CD4+ T cells was low in comparison with wild-type T cells

To examine the effect of IL-17 deficiency on T cell sensitization against the MOG peptide, we adoptively transferred CD4+ T cells into recipient mice of the same genetic background. Lymphocytes from MOG/CFA-immunized wild-type or IL-17−/− mice were stimulated with MOG peptide for 4 days in vitro, and CD4+ T cells were then purified and transferred into naive wild-type mice. The development of EAE in mice that received IL-17−/− CD4+ T cells was markedly reduced in comparison to those animals receiving wild-type CD4+ T cells (Fig. 6). These observations indicate that MOG-specific T cells from IL-17−/− mice cannot efficiently induce EAE in recipient mice.

**IL-17 production was enhanced in IFN-γ−/− mice**

MS and EAE are typically classified as Th1 cell-mediated autoimmune diseases. It has been shown, however, that the development of EAE is exacerbated in IFN-γ−/− and/or IFN-γR−/− mice (22–25), indicating that IFN-γ serves a protective role in the disease pathogenesis. Therefore, we next examined whether IFN-γ-deficiency influences IL-17 production by CD4+ T cells during EAE development. Consistent with previous reports, the LN (inguinal and axillary) cell number of IFN-γ−/− mice was significantly increased compared with that of wild-type mice 10 days after MOG/CFA immunization (Fig. 7A). When LN cells from MOG-immunized mice were cultured in the presence of MOG peptides, proliferating cells were predominantly observed in a region indicated as “R2”, while nonproliferating cells were observed in a region indicated as “R1” (Fig. 7B), as determined by CFSE labeling (data not shown). Thus, to detect MOG-reactive, IL-17-producing T cells in LN cells, cells were selectively gated to the R2 region. The percentage of IL-17-producing CD4+ T cells in the draining LN cells of IFN-γ−/− mice was greatly increased in comparison to that in wild-type mouse T cells, irrespective of MOG restimulation (Fig. 7, C and D). A large proportion of CD8+ T cells, as well as CD4+ T cells from IFN-γ−/− mice immunized with MOG/CFA, produced IL-17, although only a small proportion of CD8+ cells from wild-type mice produced IL-17 (Fig. 7, C and D).

Next, we assessed the effect of IL-17 on IFN-γ production during MOG immunization. As shown in Fig. 4, D and E, although MOG-specific T cell proliferation was impaired in IL-17−/− mice, IFN-γ levels in culture supernatants were normally observed. These observations suggested that the IFN-γ-producing cell population is increased in IL-17−/− mice. In support of this, the percentage of IFN-γ-producing CD4+ T cells in the draining LN cells...
of IL-17−/− mice was significantly increased compared with that in wild-type mice after MOG stimulation (Fig. 8). Similar results were also obtained in CD8+ T cells (Fig. 8). Thus, these data suggest that IL-17 production is regulated by IFN-γ while IFN-γ is regulated by IL-17.

Then, we examined whether IL-17 and IFN-γ can directly regulate IFN-γ and IL-17 production, respectively. When purified splenic CD4+ T cells were stimulated with plate-coated anti-CD3 mAb in the presence of various concentration of IL-12, IFN-γ production was enhanced in a dose-dependent manner (Fig. 9A). However, IL-17 did not influence IFN-γ production by CD4+ T cells in the absence or presence of IL-12 (Fig. 9, B and C). IL-23 could promote IL-17 production dose dependently (Fig. 9D), while IFN-γ did not show any effects on IL-17 production irrespective of the presence of IL-23 (Fig. 9, E and F). These observations indicate that IFN-γ or IL-17 cannot directly modulate IL-17 or IFN-γ production.

Increased MOG-specific Ab production in IL-17−/− mice during EAE

To elucidate the role of IL-17 in MOG-specific Ab production, we measured the level of anti-MOG-specific serum Abs in wild-type and IL-17−/− mice during EAE. Before immunization with MOG peptides, the level of MOG-specific IgG was very low in both wild-type and IL-17−/− mice (Fig. 10A). On day 20 after MOG/CFA immunization with PTx injection as shown in Fig. 1, the level of MOG-specific IgG in IL-17−/− mice was slightly higher than that in wild-type mice (Fig. 10A). In chronic inflammatory phases during EAE induced by MOG/CFA with PTx, the levels of MOG-specific IgG and IgG1 in IL-17−/− mice were profoundly increased compared with these in wild-type mice, although these IL-17−/− mice did not show any sign of EAE (Fig. 10). Similarly, the levels of MOG-specific IgG2a and IgG2b were also slightly, but not significantly, increased in IL-17−/− mice, while those of MOG-specific IgG3 and IgM were not different between wild-type and IL-17−/− mice (Fig. 10B). These results indicated that IL-17 has an influence upon MOG-specific Ab production by B cells.

However, our findings suggest that no correlation exists between the susceptibility and severity of EAE and the levels of anti-MOG Abs in IL-17−/− mice.

Discussion

In this study, we have demonstrated using IL-17−/− mice that IL-17 plays an important role in the development of EAE induced by MOG/CFA. We found that, upon immunization with MOG/CFA, T cell sensitization was defective in IL-17−/− mice, and CD4+ T cells from IL-17−/− mice did not induce EAE efficiently compared with wild-type T cells. These observations suggest that IL-17 plays an important role in the activation of encephalitogenic T cells during the sensitization phase of EAE. In contrast, it was recently reported that IL-17-producing CD4+ T cells enhance the...
anti-mouse IFN-

FIGURE 8. The proportion of IFN-\(\gamma\)-producing CD4\(^+\) and CD8\(^+\) T cells was increased in IL-17\(^{-/-}\) mice after MOG immunization. LN cells from mice immunized with MOG/CFA were cultured for 72 h in the presence or absence of 50 \(\mu\)g/ml MOG peptide. IFN-\(\gamma\)-producing cells were then analyzed by FACS, and the percentage of IFN-\(\gamma\)-CD3\(^+\)CD4\(^+\) or CD3\(^+\)CD8\(^+\) T cells is shown. A, Staining of intracellular IFN-\(\gamma\) in CD3\(^+\)CD4\(^+\) or CD3\(^+\)CD8\(^+\) T cells stimulated with or without MOG peptides. Shaded areas, staining with isotype-matched control Ab; bold lines, anti-mouse IFN-\(\gamma\) staining. The percentage of IFN-\(\gamma\)-positive cells (upper bold figures) and percentage of cells which were stained with an isotype-matched control IgG (lower figures) are shown. B, Each circle represents a value from an individual mouse, and the column represents the average for each group in A. □, Wild-type mice (\(n=6\)); ■, IL-17\(^{-/-}\) mice (\(n=6\)). IgG, isotype-matched control IgG staining. IFN-\(\gamma\), anti-mouse IFN-\(\gamma\) staining. †, \(p<0.05\) vs the corresponding values for control IgG staining. *, \(p<0.05\) vs the corresponding values for the cultures in the absence of MOG peptide (medium alone). ‡, \(p<0.05\) vs the corresponding values of wild-type mice. All \(p\) values were determined by the Student \(t\) test.

disease severity of EAE and that treatment with anti-IL-17 neutralizing Abs during the elicitation phase suppressed disease development (26). These observations strongly suggest that IL-17 is involved in the pathogenesis of EAE during both the sensitization and elicitation phases.

As Th1 cells, which are the major producers of IFN-\(\gamma\), infiltrate the inflamed lesions of EAE or collagen-induced arthritis (CIA) (27, 28), it was suspected that IFN-\(\gamma\) may have a pathological role in the development of these autoimmune diseases. However, administration of neutralizing Abs for IFN-\(\gamma\) leads to exacerbation of these diseases (29). The development of CIA is enhanced in IFN-\(\gamma\)^{-/-} mice and that of EAE is also exacerbated in both IFN-\(\gamma\)^{-/-} and IFN-\(\gamma\)R^{-/-} mice compared with wild-type mice (22–25). Thus, IFN-\(\gamma\) may have a protective role in these diseases, rather than a pathogenic role. Consistent with this notion, the development of EAE is also exacerbated in mice deficient for IL-12 (23, 30). In a similar manner, the severity of EAE was exaggerated in mice deficient in IL-12B2R. Interestingly, we found that the IL-17-producing T cell population was increased in IFN-\(\gamma\)^{-/-} mice in comparison to that seen in wild-type mice upon immunization with MOG/CFA (Fig. 7). Similar observations were also currently reported by other groups (32, 33). IL-17 pro-

FIGURE 9. Exogenous IFN-\(\gamma\) and IL-17 did not directly affect IL-17 and IFN-\(\gamma\) production by CD4\(^+\) T cells. CD4\(^+\) T cells from spleen of wild-type mice were stimulated with plate-coated anti-CD3 mAAb in the presence or absence of rmIL-17 or rmIFN-\(\gamma\) with or without rmIL-12 or rmIL-23 for 48 h. Then, IFN-\(\gamma\) or IL-17 levels in culture supernatants were determined by ELISA. IFN-\(\gamma\) levels in culture supernatants from CD4\(^+\) T cells: stimulated with anti-CD3 mAAb plus the indicated amount of rmIL-12 (A), rmIL-17 (B), and 1 ng/ml rmIL-12 + indicated amount of rmIL-17 (C). IL-17 levels in culture supernatants from CD4\(^+\) T cells: stimulated with anti-CD3 mAAb plus rmIL-23 (D), rmIFN-\(\gamma\) (E), or 1 ng/ml rmIL-23 + indicated amount of rmIFN-\(\gamma\) (F). Data showed the average ± SD from three mice, and a representative result from two independent experiments.

duction was also augmented in the splenocytes of IL-12R\(\beta2^{-/-}\) mice (20). These observations suggest that IFN-\(\gamma\) plays a beneficial role during the development of EAE by regulating IL-17 production. However, we demonstrated that IFN-\(\gamma\) did not directly influence IL-17 production by CD4\(^+\) T cells (Fig. 9, E and F), suggesting that the suppressive effect of IFN-\(\gamma\) on IL-17 production may be due to the suppression of the development of IL-17-producing cells. In this context, it was recently reported that IL-17-producing cells are induced by IL-23, while IL-12/IFN-\(\gamma\) suppresses the production of IL-17 (34).

We also found that IFN-\(\gamma\)-producing CD4\(^+\) and CD8\(^+\) T cells were markedly increased in IL-17\(^{-/-}\) mice stimulated with MOG peptides, although IL-17 did not show any direct effect on IFN-\(\gamma\) production by CD4\(^+\) T cells (Fig. 9, B and C) (Fig. 8). These observations suggest that IL-17 negatively regulates the development of IFN-\(\gamma\)-producing Th1 cells. Thus, IL-17 and IFN-\(\gamma\) may mutually regulate the development of these cytokine producer cells during immune responses.

We demonstrated that CD4\(^+\) T cells are the predominant producers of IL-17 in LN cells after immunization with MOG/CFA. It has been reported that, in Lyme arthritis, IL-17 is primarily produced by a specific subpopulation of CD4\(^+\) T cells that are neither Th1 nor Th2 and that produce TNF-\(\alpha\) and/or GM-CSF simultaneously. As IL-17 is produced by multiple cell types, including CD8\(^+\) T cells, \(\gamma\)\(\delta\) T cells, neutrophils, and eosinophils under different conditions (13–18, 35), the production of IL-17 is not limited to a specific T cell population. Instead, the producer cells in a particular disease appear to be defined by a specific cell population. The mechanism by which these IL-17 producer cells are controlled in different diseases, however, remains to be elucidated.

In MS patients, elevation of anti-MOG Ab levels is detectable in cerebrospinal fluid (36, 37). In association with the elevation of
between the severity of EAE and the elevation of anti-MOG Abs development of EAE was markedly suppressed in IL-17 seen in wild-type mice after immunization with MOG, while the mechanism. Additional experiments will be necessary to elucidate the molecular nature of the Ags involved in the autoimmune disorders, 11). We do not currently understand the reasoning for this. The CIA and contact, delayed-type, and airway hypersensitivity (9 – 11). Nakae, S., A. Nambu, K. Sudo, and Y. Iwakura. 2003. Suppression of immune responses are clearly different; EAE and CIA are IL-17 dependent, and IL-17-producing cells play a major role, while IDDM and GVHR are IFN-γ dependent, and CD8+ cytotoxic T cells and/or CD4+ Th1 cells play important roles.

Taken together, our data demonstrate that IL-17 and IFN-γ, produced by a distinct population of T cells, have different roles in the development of EAE, CIA, GVHR, and hyperglycemia. These results suggest that these cytokines may also be involved in the development of MS, RA, GVHD, and IDDM in humans. Elucidation of the roles of pathogenic cytokines and the mechanisms of cytokine dependency may provide potential targets for novel therapeutics to treat these diseases.

**Acknowledgments**

We thank Dr. S. J. Gali (Stanford University School of Medicine, Stanford, CA) for his generous support for this study. We also thank Tomoko Hata and Hayato Kotaki for their excellent animal care.

**Disclosures**

The authors have no financial conflict of interest.

**References**


