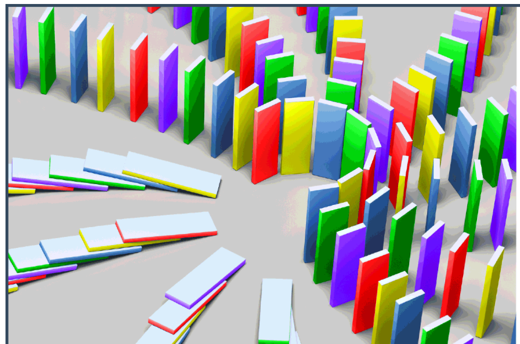

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Anti-Inflammatory Role of IL-17 in Experimental Autoimmune Uveitis[1]

Yan Ke,* Ke Liu,‡ Guo-Qiang Huang,‡ Yan Cui,† Henry J. Kaplan,* Hui Shao,[2,3]* and Deming Sun[2,3]†

Previous studies have shown that IL-17 is a strong proinflammatory cytokine and that IL-17-producing autoreactive T cells play a major role in the pathogenesis of autoimmune diseases. In a previous study, we showed that injection of experimental autoimmune uveitis-susceptible mice with anti-IL-17 Abs blocked subsequent disease development. To determine whether administration of IL-17 to experimental autoimmune uveitis-susceptible Lewis rats and B10RIII mice injected with disease-inducing peptides enhanced disease susceptibility, we injected the recipient animals with various doses of human rIL-17 (hIL-17). Unexpectedly, the treated animals showed significant amelioration of disease; in addition, both the intensity of the autoreactive response and cytokine production by the autoreactive T cells induced by immunization with uveitogenic peptides were significantly decreased. Our results show that IL-17 has anti-inflammatory activity and that this cytokine can suppress the development of autoimmune disease. ***The Journal of Immunology,* 2009, 182: 3183–3190.**

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Recent studies have identified an autoreactive T cell subset that produces IL-17, but not IFN-γ and IL-4 (1, 2). Other studies have demonstrated that IL-17-producing T cells are crucially involved in the pathogenesis of autoimmune diseases, such as rheumatoid arthritis, experimental autoimmune encephalomyelitis (3–5), and other allergic diseases (6–8). This is supported by the observations that IL-17-deficient mice are resistant to an arthritis-like disease (9, 10) and have impaired host defense against microbial infection and an increased incidence of acquired delayed-type hypersensitivity (9). In addition, autoimmune disease-susceptible mice become disease resistant after treatment with an IL-17R antagonist (11).

Previous studies have shown that IL-17 is functionally proinflammatory. It increases the local production of chemokines and promotes recruitment of monocytes and neutrophils (12–16). It also directly activated fibroblasts and synoviocytes, leading to the production of IL-6, IL-8, PGE_2, and G-CSF, thus enhancing the local inflammatory environment (17–20). In addition, it drives T cell responses, notably through induction of the costimulatory molecule ICAM (17, 21, 22). During inflammatory conditions, IL-17 has an enhancing effect on NO production by cartilage (23).

In a previous report, we showed that IL-17^+ interphotoreceptor retinoid-binding protein (IRBP)[4] peptide-specific T cells play a major role in the pathogenesis of experimental autoimmune uveitis (EAU) and that injection of anti-IL-17 Abs inhibits the development of disease (24). We also showed that, while adoptive transfer of a small number of IL-17^+ IRBP peptide-specific T cells induces EAU, transfer of a larger number of Ag-nonspecific IL-17^+ T cells does not (24). We therefore wished to determine the separate pathogenic role of IL-17 and IL-17^+ autoreactive T cells. This report shows that, unexpectedly, injection of EAU-susceptible rats or mice with small doses of IL-17 ameliorated, rather than exacerbated, disease development. In addition, IL-17-treated rats had a significantly lower T cell response to the immunizing Ag and T cells isolated from IL-17-treated rats had a suppressor effect on EAU development on adoptive transfer into EAU-susceptible rats. We therefore conclude that IL-17 has both pro- and anti-inflammatory effects on the development of EAU.

Materials and Methods

Animals and reagents

Female Lewis rats (6- 8 wk old) and B10RIII mice (12–14 wk old) were purchased from The Jackson Laboratory and were housed and maintained in the animal facilities of the University of Louisville or the Doheny Eye Institute at the University of Southern California. Institutional approval was obtained and institutional guidelines regarding animal experimentation were followed. All T cells were cultured in RPMI 1640 medium (Life Technologies) supplemented with 10% FCS (Life Technologies), 5×10^{-5} M 2-ME, and 100 μg/ml penicillin/streptomycin. IFA was obtained from Sigma-Aldrich. CFA in the text refers to IFA with added *Mycobacterium tuberculosis* H37Ra (Difco). IRBP peptides 1177–1191 (ADGSSWEGVGVVPDV, R16) and $IRBP_{161–180}$ (SGIPYIISYLHPGNTILHVD) were synthesized by Sigma-Aldrich. hIL-17 (hIL-17) was a gift from the Research Group of Southern Biotechnology Associates. FITC-conjugated anti-mouse IL-17 and PE-conjugated anti-rat IFN-γ, anti-rat Foxp3 Abs were obtained from eBioscience.

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Received for publication July 28, 2008. Accepted for publication December 18, 2008.

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[1] This work was supported in part by National Institutes of Health Grants EY12974, EY14599, EY014366, and EY017373. D.S. is a recipient of a senior investigator award from Research to Prevent Blindness. H.S. is a recipient of a career development award from Research to Prevent Blindness.

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[4] Abbreviations used in this paper: IRBP, interphotoreceptor retinoid-binding protein; EAU, experimental autoimmune uveitis; hIL-17, human IL-17; p.i., postimmunization; PMN, polymorphonuclear neutrophil.

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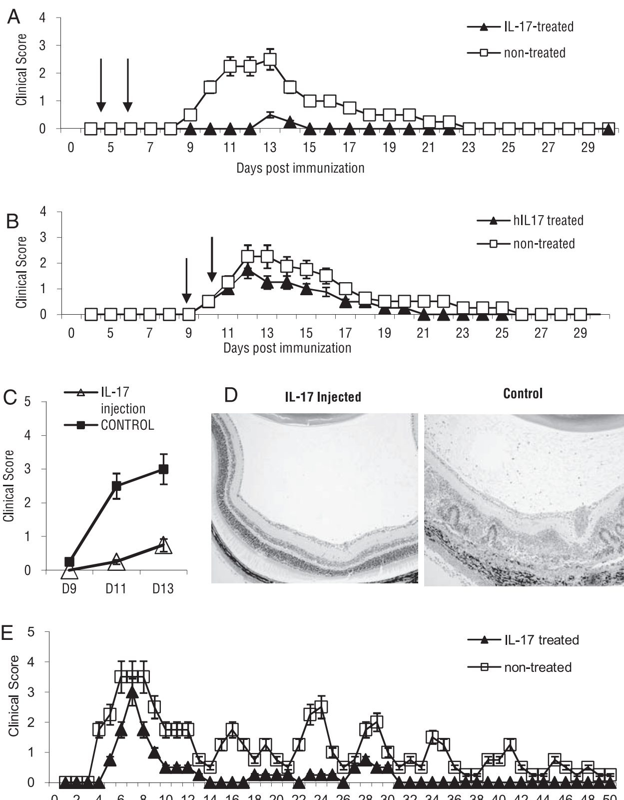

FIGURE 1. Injection of Lewis rats and B10RIII mice with small doses of IL-17 significantly ameliorates the development of EAU. *A* and *B*, R16-immunized Lewis rats received two i.p. injections of hIL-17 (3 μg/injection) on days 4 and 7 (*A*) or days 10 and 13 (disease onset) p.i. (*B*). Disease severity was observed using a slit lamp microscope and graded using previously described criteria. The mean score at each time point is the average for six animals. *C* and *D*, $IRBP_{161-180}$-immunized B10RIII mice received three i.p. injections of IL-17 (1 μg/injection) on days 0, 4, and 8. Disease severity was monitored by fundoscopy on days 9, 11, and 13 (*C*) and pathologic examination was performed on day 13 (*D*). *E*, T cells isolated at day 13 p.i. from the draining lymph nodes and spleens of R16-immunized Lewis rats with or without IL-17 treatment on days 4 and 7 were enriched by passage through nylon wool and restimulated in vitro with R16 (10 μg/ml) presented by irradiated syngeneic spleen APCs. After 3 days, activated T cell blasts were separated on a Ficoll gradient and injected (3×10^6cells, i.v.) into naive Lewis rats. Clinical signs were observed by slit lamp biomicroscopy and scored as described in *Materials and Methods*. The results shown are the mean ± SD for three animals in a single experiment, which was repeated three times with similar results.

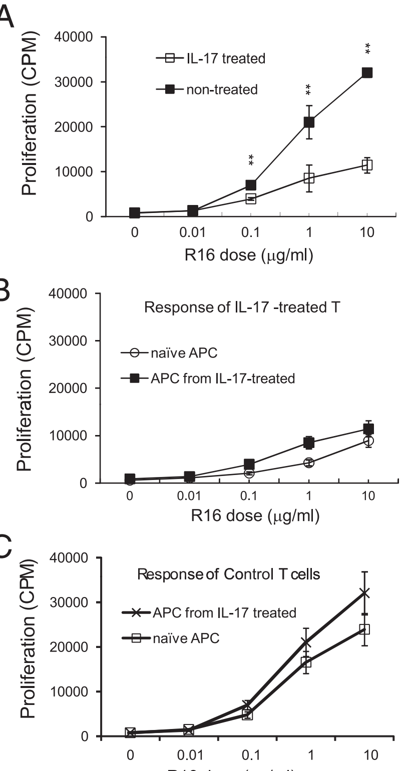

FIGURE 2. hIL-17-treated R16-immunized rats have a decreased T cell response to the immunizing peptide. *A*, Two groups of R16-immunized rats were treated with or without hIL-17 on days 4 and 7, then, on day 10, enriched splenic T cells were pooled and tested in 96-well plates for their proliferative response in the presence of graded doses of R16 and irradiated APCs from naive rats. *B* and *C*, The responder T cells from *A* from hIL-17-treated (*B*) or nontreated (*C*) R16-immunized rats were incubated with irradiated spleen cells from either naive or IL-17-treated rats and their proliferative response to Ag stimulation was measured. The proliferative response is expressed as the mean cpm ± SD of triplicate determinations and the result is representative of those for three separate experiments; the SD was <15%. Values of $p < 0.05$ were labeled with * and those <0.01 were labeled with **.

Actively induced and adoptively transferred uveitis

The active induction of uveitis in Lewis rats using peptide $IRBP_{1177-1191}$ (R16) has been previously described (25). Briefly, the rats were immunized s.c. with 200 μl of an emulsion containing 50 μg of R16 and 500 μg of *Mycobacterium tuberculosis* H37Ra (Difco) in IFA, distributed over six spots on the tail base and flank. The animals were observed using a slit lamp biomicroscope for clinical signs of uveitis. The EAU clinical score was the average for all of the rats in the group, with each rat (average of both eyes) being treated as one statistical event. The intensity of the clinical signs was scored on an arbitrary scale of 0–4, with 0 = normal; 1 = engorged blood vessels in the iris, abnormal pupil contraction, and turbidity of the aqueous humor in the anterior chamber; 2 = hazy anterior chamber; 3 = moderately opaque anterior chamber, but still visible pupil; and 4 = opaque anterior chamber and obscured pupil and proptosis. Overt clinical signs usually began 8-12 days postimmunization and subsided within 5-10 days. The inflammation began in the anterior segment, then spread to the retina, with minimal involvement of the choroid. No spontaneous recurrence of EAU occurred. For adoptive transfer, recipient rats (5–6 wk old) were injected i.v. with 2×10^6 R16-specific T cells in 0.2 ml of PBS.

Induction of EAU in the B10RIII mouse was performed using our previously reported procedures (26, 27). Briefly, the mice were immunized s.c. with 200 μl of an emulsion containing 50 μg of $IRBP_{161-180}$ and 500 μg of *M. tuberculosis* H37Ra (Difco) in IFA, distributed over six spots on the tail base and flank. The animals were examined three times a week for clinical signs of uveitis by fundoscopy, starting at week 2 after transfer. Fundoscopic evaluation for longitudinal follow-up of disease was performed using a binocular microscope after pupil dilation using 0.5% tropicamide and 1.25% phenylephrine hydrochloride ophthalmic solutions. The incidence and severity of EAU were graded on a scale of 0–4 in half-point increments using previously described criteria (28) based on the type, number, and size of lesions present.

Histology and immunohistochemistry

For histology, whole eyes were collected at the end of the experiment (days 11–20 after immunization) and prepared for histopathological evaluation. The eyes were immersed for 1 h in 4% phosphate-buffered glutaraldehyde, then transferred to 10% phosphate-buffered formaldehyde until processed. Fixed and dehydrated tissues were embedded in methacrylate and 5- μm sections were cut through the pupillary-optic nerve plane. Sections were stained with H&E. The presence or absence of disease was evaluated blind by examining six sections cut at different levels for each eye. Severity of EAU was scored on a scale of 0 (no disease) to 4 (maximum disease) in half-point increments based on the presence of inflammatory cell infiltration of the iris, ciliary body, anterior chamber, and retina, where 0 =

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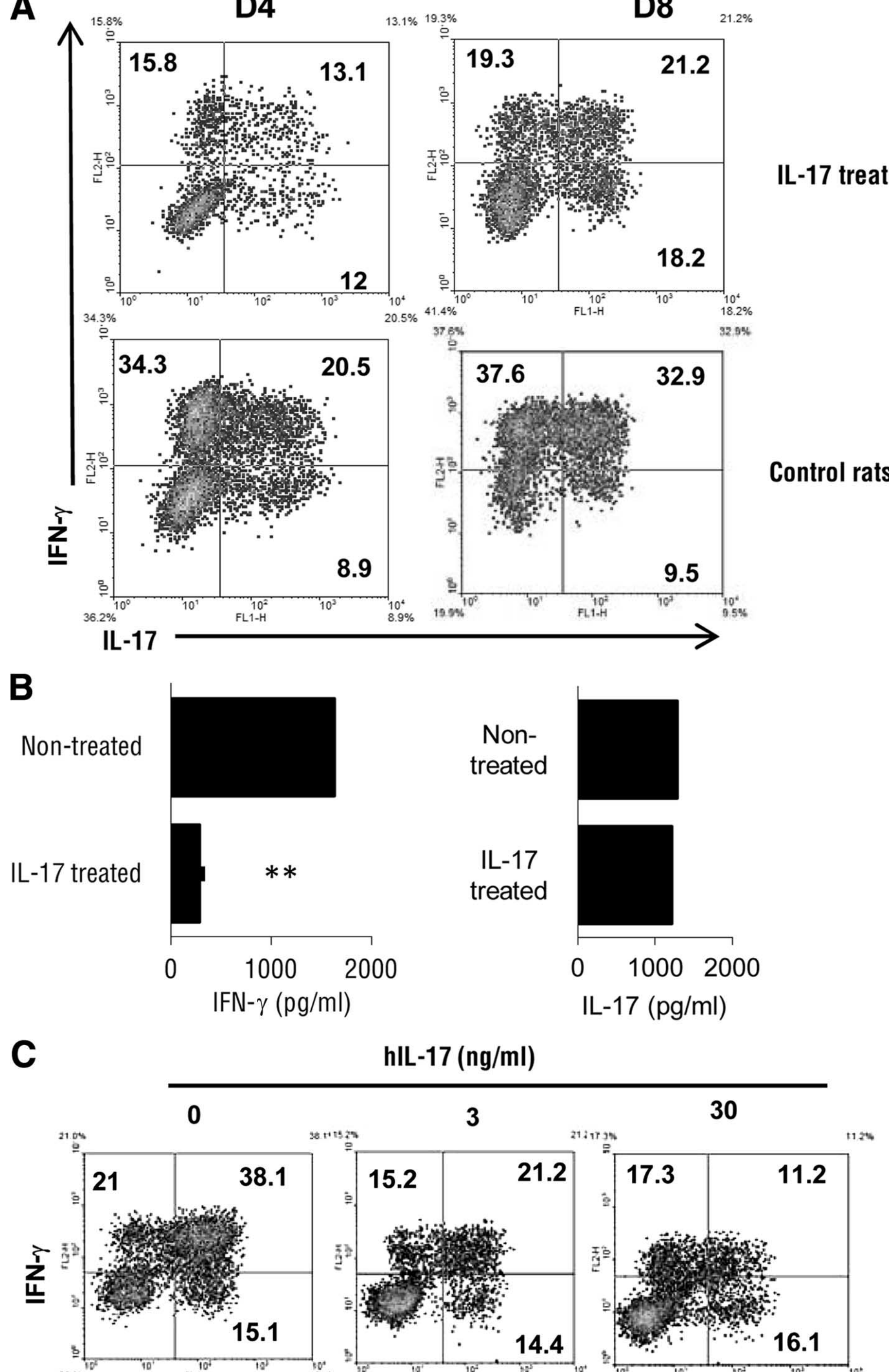

FIGURE 3. IL-17 treatment preferentially inhibits the activation of IFN-γ^+ IRBP-specific T cells. *A*, Enriched T cells from the spleen and draining lymph nodes of R16-immunized rats with or without IL-17 treatment were incubated with the immunizing peptide for 2 days in the presence of syngeneic APCs and the activated T cells separated by Ficoll gradient centrifugation and continuously cultured in cytokine-free medium for 2, 4, or 6 days, then intracellular expression of IFN-γ and IL-17 was assessed by FACS analysis. *B*, Enriched T cells from spleen and draining lymph nodes of R16-immunized rats with or without IL-17 treatment were incubated with the immunizing peptide for 2 days in the presence of syngeneic APCs, then IFN-γ and IL-17 production was measured by ELISA. *C*, Enriched T cells from R16-immunized rats not treated with IL-17 were stimulated for 48 h with R16 and APCs in the presence of graded amounts (0, 3, or 30 ng/ml) of hIL-17, then the activated T cells in each group were intracellularly stained with anti-IFN-γ and anti-IL-17 Abs, followed by FACS analysis. The results shown are representative of those in three experiments.

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normal anterior segment and retinal architecture, with no inflammatory cells in these structures; 1 = mild inflammatory cell infiltration of the anterior segment and retina; 2 = moderate inflammatory cell infiltration of the anterior segment and retina; 3 = massive inflammatory cell infiltration of the anterior segment and retina, disorganized anterior segment and retina; and 4 = as in 3, but with photoreceptor cell damage.

Immunofluorescence flow cytometry

Aliquots of 1×10^6 cells were incubated for 30 min at 4°C with anti-CD4 or isotype control Abs, fixed overnight with 1 ml of fixation buffer, washed, and incubated for 30 min at 4°C with anti-rat Foxp3 Abs.

For intracellular cytokine staining, cells were pretreated for 4 h with 50 ng/ml PMA, 1 μg/ml ionomycin, and 1 μg/ml brefeldin A (Sigma-Aldrich), then washed, fixed, permeabilized overnight with Cytofix/Cytoperm buffer (eBioscience), and intracellularly stained with Abs against IFN-γ and IL-17. Data collection and analysis were performed on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences).

T cell proliferation assays

APCs (irradiated syngeneic spleen cells, 2×10^5/well) were preincubated for 1 h in 96-well flat-bottom microtiter plates with an optimal dose (5 μg/ml) of R16 in the absence or presence of IL-17, IFN-γ, or LPS, then enriched T cells (4×10^4/well), prepared from lymph nodes or spleen by nylon wool adhesion, were added and incubation was continued for 48–72 h. [^{3}H]Thymidine incorporation during the last 16 h was assessed using a

microplate scintillation counter (Packard). The proliferative response was expressed as the mean cpm ± SD of triplicate determinations.

ELISA

IL-17 and IFN-γ were measured using commercially available ELISA kits (R&D Systems).

Statistical analysis

The data are expressed as the mean ± SD of the results for at least three separate experiments. Statistical analyses were performed using Student's *t* test. A *p* value of 0.05 or less was considered to be statistically significant.

Results

hIL-17 treatment protects rats against uveitis induced by R16 injection

Lewis rats immunized with 50 μg of R16 emulsified in CFA develop uveitis after 8–9 days. To determine whether hIL-17 had an effect on R16-induced uveitis, R16-injected rats received two i.p. injections of hIL-17 (3 μg/injection) on days 4 and 7 after R16 injection (day 0), while control rats received PBS. Although all control animals developed full disease, hIL-17-treated rats developed mild disease, with a delayed onset (Fig. 1*A*). We then determined whether delayed injection of hIL-17 had a similar protective effect. As shown in Fig. 1*B*, two injections of the same dose starting at disease onset (days 10 and 12) did not significantly affect EAU development, indicating that early treatment is required for a therapeutic effect.

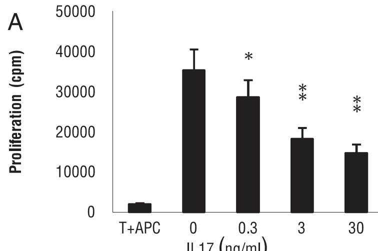
To determine whether hIL-17 also affected the development of mouse EAU, B10RIII mice were immunized with 200 μg of $IRBP_{161-180}$ peptide emulsified in CFA with or without IL-17 treatment. Mice subjected to hIL-17 treatment received three i.p. injections of hIL-17 (1 μg/mouse) on days 0, 4, and 8. The animals were examined every 2 days for clinical signs of uveitis by fundoscopy, starting on day 8 after immunization. The results showed that, although all control animals developed full disease, hIL-17-treated mice were protected (Fig. 1, *C* and *D*).

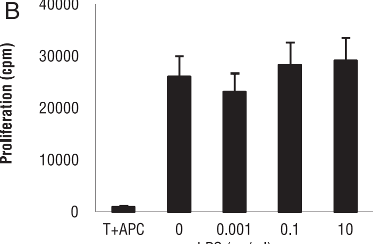
Effect of IL-17 treatment on chronic relapsing uveitis

We previously reported that adoptive transfer of R16-specific T cells into naive Lewis rats induces chronic relapsing disease in contrast to the monophasic disease induced by immunization with R16 emulsified in CFA (29). To determine whether relapsing disease was similarly affected by IL-17 treatment, recipient rats underwent adoptive transfer of in vitro-activated R16-specific T cells (3×10^6/rat) from in vivo- primed syngenic rats and IL-17 (3 μg/injection) was administered on days 0 and 3 after T cell transfer. Compared with rats treated with PBS, the IL-17-treated rats ($n = 6$) developed much milder relapses, both in terms of the number of relapses and the intensity of ocular inflammation (Fig. 1*E*).

Decreased activation of IRBP-specific T cells in immunized rats as a result of hIL-17 treatment

To determine the mechanism by which hIL-17 injection prevents the development of uveitis, we measured R16-specific T cell responses in untreated and hIL-17-treated R16-immunized animals. Groups ($n = 3$) of rats immunized with a uveitogenic dose of R16 (50 μg) with or without hIL-17 treatment on days 4 and 7 were sacrificed on days 10–15 and a splenic T cell-enriched preparation was prepared by nylon wool adhesion and seeded (4×10^5/well) in 96-well plates to test proliferation in the presence of graded doses of R16 and irradiated APCs from naive rats. As shown in Fig. 2*A*, hIL-17-treated rats showed greatly decreased T cell proliferation. To exclude the possibility that the low responsiveness of T cells seen in hIL-17-treated animals resulted from dysfunction of APCs, we performed cross-tests in which responder T cells derived from hIL-17-treated or control-treated rats were mixed with irradiated APCs from either naive or hIL-17-treated rats. As shown in Fig. 2, *B* and *C*, the hIL-17-treated rats had a low R16-specific T cell response regardless of the origin of the APCs, and the APC function of IL-17-treated animals was not appreciably altered.





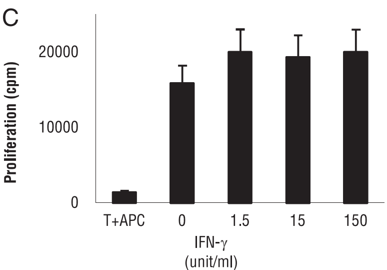


FIGURE 4. IL-17 has an in vitro inhibitory effect on the activation of IRBP-specific T cells. T cells (4×10^5/well) prepared from the spleens of R16-immunized rats (9 days p.i.) were stimulated in 96-well plates with R16 (10 μg/ml) and APCs for 48 h in the presence of graded doses of recombinant hIL-17 (*A*), LPS (*B*), or mouse IFN-γ (*C*) and T cell proliferation was assessed at 48 h by [^{3}H]thymidine uptake. Values of $p < 0.05$ were labeled with * and those <0.01 were labeled with **.

IL-17 treatment has a stronger effect inhibiting the IFN-γ^+ IRBP-specific T cells

To determine whether IL-17 treatment affected the activation of IFN-γ and/or IL-17^+ autoreactive T cells, R16-specific T cells were prepared from the spleens and draining lymph nodes of R16-immunized rats at day 13 postimmunization (p.i.) and stimulated in vitro by immunizing Ag (10 μg/ml) and APCs (irradiated spleen cells) for 2 days, then activated T cell blasts were separated by Ficoll gradient centrifugation and continuously cultured in cytokine-free medium for 2 or 6 days (a total of 4 or 8 days in culture), then stained intracellularly with FITC-anti-IL-17 and PE-anti-IFN-γ Abs. As shown in the *lower left panel* of Fig. 3*A*, 4 days after the end of in vitro stimulation with the immunizing Ag, 54.8% of the T cells expressed IFN-γ and 29.4% expressed IL-17, with 20% of the cells expressing both IFN-γ and IL-17. The percentage of IFN-γ^+- and IL-17^+ cells increased gradually over the subsequent days of culture in vitro (Fig. 3*A*, *right panel*). In contrast, T cells from IL-17-treated rats only contained 28.9% IFN-γ^+

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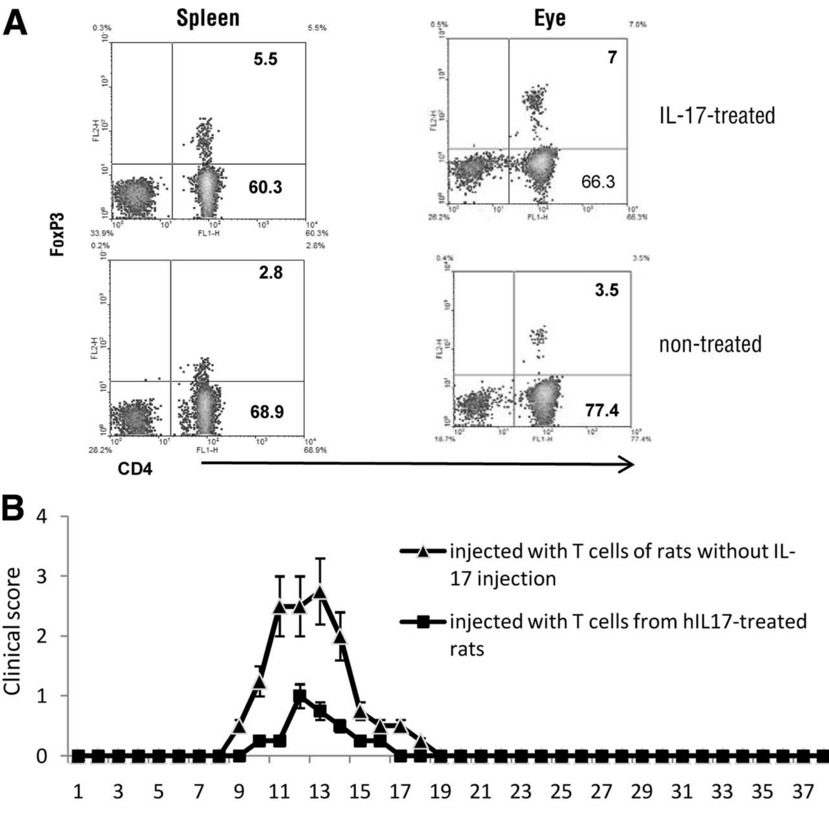

FIGURE 5. hIL-17 treatment results in enhanced regulatory T cell activity. *A*, Rats treated with IL-17 express increased numbers of $Foxp3^+$ T cells in the periphery and in the inflamed eye. Lewis rats were immunized with R16 with or without IL-17 treatment (two i.p. injections of 3 μg/injection on days 4 and 7), then splenic T cells and eye-infiltrating cells were prepared on day 10 p.i. and the number of $CD4^+Foxp3^+$ T cells were assessed by dual staining with anti-CD4 and anti-Foxp3 Abs and FACS analysis. *B*, Splenic T cells from hIL-17-treated rats have increased suppressor activity. Two groups ($n = 4$) of rats were immunized with a pathogenic dose of R16 (50 μg/rat) and one group was also injected i.p. with 50×10^6 splenic T cells isolated on day 15 from R16-immunized rats treated with IL-17 on days 4 and 7 or from R16-immunized rats without IL-17 treatment (control). Disease was monitored and scored. The results shown are the mean $\pm$ SD from one representative experiment of two.

and 25.1% $IL\text{-}17^+$ cells on day 4 in culture (Fig. 3*A*, *upper left panel*). The number of $IFN\text{-}\gamma^+$ T cells in the IL-17-treated rats decreased more significantly with time than the number of $IL\text{-}17^+$ T cells. The results of a cytokine assay agreed with the intracellular staining results, as T cells from IL-17-treated animals produced significantly lower amounts of IFN-γ than T cells from control animals, whereas IL-17 production was only marginally decreased (Fig. 3*B*).

Similar results were also seen in an in vitro study. When IL-17 was added to cultures of R16-specific T cells from non-IL-17-treated rats incubated with R16 and APCs for 48 h, it inhibited the activation of $IFN\text{-}\gamma^+$ R16-specific T cells in a dose-dependent manner, but did not affect the activation of Th17 cells (Fig. 3*C*). The proliferative response of R16-specific T cells was dose-dependently inhibited by in vitro IL-17 treatment (Fig. 4*A*), but not by LPS (Fig. 4*B*) or IFN-γ (Fig. 4*C*).

IL-17 administration induces enhanced regulatory T cell activity

One reason why IL-17 treatment decreases EAU susceptibility in recipient animals could be that it promotes the expansion of T cells with regulatory activity or enhanced regulatory T cell activity. We therefore assessed the number of $Foxp3^+$ T cells in R16-injected rats with and without IL-17 treatment. As shown in Fig. 5*A*, the treated rats had significantly increased numbers of $Foxp3^+$ T cells in T cells isolated from the spleen or the inflamed eye.

We also tested whether regulatory T cell activity was increased in the IL-17-treated animals. Splenic T cells were prepared from IL-17-treated or untreated rats immunized with peptide R16 on day 10 p.i. and 5×10^7 T cells were adoptively transferred to recipient rats immunized with a pathogenic dose of R16 emulsified in CFA. As shown in Fig. 5*B*, the recipient rats that received T cells from IL-17-treated rats developed much weaker disease, with a delayed onset, compared with those that received T cells from untreated rats.

Discussion

Recent studies have identified a unique CD4 T cell subset that expresses IL-17, but not IFN-γ or IL-4 (1, 2, 30) and have demonstrated that such T cells play a major role in the pathogenesis of autoimmune diseases (31–33). Previous studies have shown that the biological actions of IL-17 are proinflammatory. IL-17 increases the local production of chemokines (12–16), thereby promoting the recruitment of monocytes and neutrophils. Furthermore, IL-17 stimulates the production of the hematopoietic cytokines G-CSF and GM-CSF, which promote the expansion of these myeloid lineages (34, 35). In addition, it stimulates the production of IL-6 and PGE_2, thus enhancing the local inflammatory environment (18–20). IL-17 also drives T cell responses, notably through induction of the costimulatory molecule ICAM (17, 21, 22).

In a previous report, we demonstrated the existence and pathogenic role of IL-17-producing autoreactive T cells in EAU (24, 36). Our studies have also shown that, although IRBP-specific $IL\text{-}17^+$ T cells are uveitogenic, $IL\text{-}17^+$ T cells expanded by anti-CD3 stimulation and thus Ag-nonspecific are not, suggesting that at least a part of the pathogenic activity of $IL\text{-}17^+$ autoreactive T cells is not mediated by their ability to produce IL-17.

To distinguish between the role of a direct cellular effect and the effect of the cytokines produced by $IL\text{-}17^+$ autoreactive T cells in their pathogenic and immunoregulatory activity, we examined whether administration of exogenous IL-17 to disease-susceptible mice promoted disease development and unexpectedly found that treatment of rats injected with IRBP-inducing

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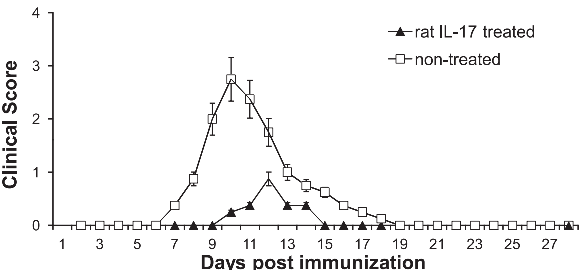
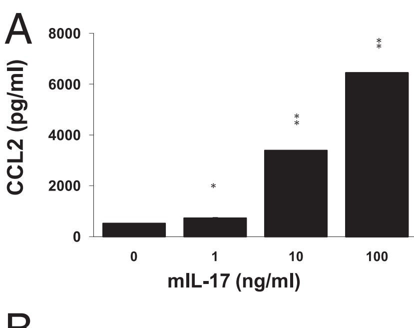


FIGURE 6. Protective effect of rat IL-17 on rat EAU. R16-immunized Lewis rats received two i.p. injections of rIL-17 (3 μg/injection) on days 4 and 7. Disease severity was observed using a slit lamp microscope and graded using previously described criteria. The results shown are the mean ± SD for three animals in a single experiment, which was repeated two times with similar results.

peptide with recombinant hIL-17 significantly inhibited the development of EAU, rather than promoted disease development. This observation was supported by results from EAU-susceptible B10RIII mice showing that IL-17-treated mice showed significantly reduced disease susceptibility. Our results show that IL-17 has an anti-inflammatory effect in the development of EAU. In our initial studies, we used a IL-17 recombinant protein of human origin, due to that most of these studies used a rat EAU model and the recombinant rat IL-17 was not yet commercially available. With the recent availability of rat IL-17, we repeated the studies also using rat IL-17. The results reproduced well with those that used hIL17 (Fig. 6). In addition, hIL-17 has an effect on murine cells (stimulation of fibroblasts to express ICAM-1 and produce cytokines/chemokines) and has been reported in previous studies (37, 38). Our own studies have also shown that hIL-17 induces chemokine production by rat and mouse macrophages in vitro (Fig. 7).

It is interesting to note that hIL-17 treatment ameliorated both forms of uveitis induced by Ag immunization or by adoptive transfer of R16-specific T cells. We have previously shown that, although immunization with an uveitogenic peptide induces acute and monophasic EAU in Lewis rats, adoptive transfer of a pathogenic dose of isolated uveitogenic T cells induces chronic and relapsing disease (29, 39). To determine whether IL-17 treatment affected the progression of EAU, rats induced to show relapsing EAU were injected with IL-17. Interestingly, the IL-17-treated animals showed little change in disease onset, but the number of relapses was significantly decreased, suggesting that IL-17 treatment has a long-term effect that persists after the end of treatment.



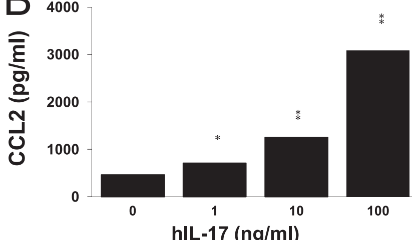


FIGURE 7. hIL-17 induces chemokine production by mouse macrophages. Mouse macrophages were cultured from bone marrow cells by medium containing M-CSF (61, 62). Monolayers of macrophages cultured in 24-well plate were exposed to graded doses of mouse IL-17 (mIL-17) or hIL-17 as indicated. Forty-eight hours later, the culture supernatants were assessed for production of CCL2 using ELISA. Values of $p < 0.05$ were labeled with * and those <0.01 were labeled with **.

The cellular and molecular basis for the protective effect of IL-17 in this autoimmune model remains to be determined. Several possible mechanisms might be considered. Since membrane IL-17 regulates the migration of polymorphonuclear neutrophils (PMNs) in the spleen (40), it was possible that i.p. injection of IL-17 sequesters the PMNs in the injection site in the peritoneum, which may affect the development of inflammation. To examine this possibility, we examined peritoneal exudate cells in IL-17-injected mice and found that i.p. injection of IL-17 did not cause significant inflammation and PMN recruitment in the peritoneum (data not shown).

That a specific cytokine possesses both pro- and anti-inflammatory activities is not a surprising observation. For example, an anti-inflammatory effect of the classic proinflammatory cytokine TNF-α has been reported (41–43). A dual role of TNF-α in type 1 diabetes has also been observed (44). Likewise, IFN-γ has been found to be both proinflammatory (45, 46) or anti-inflammatory (47–51) and IL-6 has both anti-inflammatory (52–54) and proinflammatory (55–57) effects (56, 58–60).

It is interesting to note that a given cytokine tends to be proinflammatory during the acute phase and anti-inflammatory during the chronic phase of the same autoimmune disease (49). However, it would be of great interest to test the possibility that, while IL-17$^+$ autoreactive T cells are pathogenic, the IL-17 produced by such T cells may exert a suppressive effect. The continuation of the study should allow us to reveal the complexity of immunoregulation.

Unfortunately, due to the limited amount of recombinant protein, we were unable to test whether larger doses of IL-17 tend to be proinflammatory and smaller doses immunosuppressive. In addition, it would also be of interest to test whether administration of IL-17 at different phases of an autoimmune disease has different clinical effects. In summary, our results document the immunosuppressive effect of IL-17 and call for a more thorough investigation of the biological function of IL-17 before approaches targeting this cytokine are applied in therapeutic trials.

Disclosures

The authors have no financial conflict of interest.

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