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*J Immunol* published online 11 June 2014
http://www.jimmunol.org/content/early/2014/06/10/jimmunol.1400931

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IL-17 Promotes Murine Lupus

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The proinflammatory activity of IL-17–producing Th17 cells has been associated with the pathogenesis of several autoimmune diseases. In this article, we provide direct evidence for a role of IL-17 in the pathogenesis of systemic lupus erythematosus (SLE). The induction of SLE by pristane in IL-17–sufficient wild-type mice did not occur in IL-17–deficient mice, which were protected from development of lupus autoantibodies and glomerulonephritis. The protection from SLE in IL-17–deficient mice was associated with a reduced frequency of CD3+CD4+CD8− double-negative T cells and an expansion of CD4+ regulatory T cells, and did not depend on Stat-1 signaling. These data affirm the key role of IL-17 in the pathogenesis of SLE and strengthen the support for IL-17 blockade in the therapy of SLE. The Journal of Immunology, 2014, 193: 000–000.

Materials and Methods

Mice

Animals were used at 8 wk of age. Wild-type (WT) C57BL/6 (B6) mice were from The Jackson Laboratory (Bar Harbor, ME), and syngeneic IL-17–deficient (IL-17−/−) mice were kindly provided by Dr. Yoichiros, University of Tokyo). Double-knockout IL-17−/−/Stat1−/− mice were obtained from intercross of F1 heterozygotes that had been obtained from breeding IL-17−/− mice with Stat1−/− mice (Taconic, Hudson, NY). Experimental mice received one i.p. injection of 500 μl pristane (Sigma-Aldrich, St. Louis, MO) or saline as control (10). Mice were treated in conformity with federal and institutional guidelines on animal welfare, and were fed regular chow diet in a temperature-controlled room with a 12-h light/dark cycle.

Flow cytometry

Phenotypic analyses were performed with combinations of fluorochrome-conjugated Ab using standard techniques. After Fc blocking, cells were labeled with FITC-, PE-, PerCP-, or allophycocyanin-conjugated anti-mouse Ab to CD3 (clone 17A2), CD4 (clone RM4-5), CD8 (clone 53-6.7), or CD25 (clone PC61.5) for surface staining, and to Foxp3 (clone FJK-16s) labeled with FITC-, PE-, PerCP-, or allophycocyanin-conjugated Ab using standard techniques. After Fc blocking, cells were

Histology

Kidney sections (4 μm) were stained with H&E according to standard procedures. Assessments of pathologic changes included glomerular activity score and tubulointerstitial activity score, which were measured as described elsewhere (12), in a blinded fashion. For indirect immunofluorescence analyses, sections were fixed in cold acetone for 5 min, washed, and blocked with 3% BSA for 1 h before staining with the following FITC-conjugated Abs: rabbit anti-mouse IgG, rabbit anti-mouse IgM, or goat anti-mouse C3 (all from Fisher Scientific, Waltham, MA). Sections were counterstained with H&E for histologic evaluation.

Statistical analyses

Statistical analyses were performed with Prism 5 software (GraphPad, San Diego, CA) using the paired t test or Mann–Whitney U test. The p values <0.05 were considered significant.
Results

IL-17 deficiency protects mice from serologic manifestations of SLE

Total IgG, anti-ssDNA, anti-nRNP, and anti-chromatin IgG levels were monitored in IL-17−/− and control (WT) mice after treatment with the lupus-inducing agent pristane (10). Compared with pristane-treated WT controls that started to develop hypergammaglobulinemia and autoantibodies by 12 wk posttreatment (Fig. 1), pristane-treated IL-17−/− mice had significantly reduced titers of IgG and low anti-ssDNA, anti-nRNP, and anti-chromatin autoantibodies (Fig. 1). Total IgG and autoantibody levels were comparable in control groups of WT and IL−17−/− mice treated with vehicle (Fig. 1). Anti-dsDNA autoantibody levels were not measurable at any time point in IL-17−/− mice (with or without pristane treatment) but were detectable in WT mice at 20 wk after pristane treatment (data not shown), indicating that although induction of anti-ssDNA, anti-nRNP, and anti-chromatin autoantibodies was IL-17 dependent, the induction of anti-dsDNA Ab was not.

IL-17 deficiency prevents lupus nephritis

IL 17−/− mice and WT mice were sacrificed 20 wk after pristane treatment. Kidney histopathology showed that pristane-treated IL-17−/− mice had significantly lower renal damage than pristane-treated WT mice, as indicated by the glomerular and tubulointerstitial activity scores, and the lack of immune complexes (IgG, IgM) and C3 deposition (Fig. 2).

The findings of protection from the development of autoantibodies in IL-17−/− mice treated with pristane (Fig. 1), together with the reduced kidney pathology (Fig. 2), suggest that IL-17 promotes not only the development of serologic manifestations of SLE, but also renal injury.

Protective effects of IL-17 deficiency do not depend on Stat1

IL-17 deficiency is associated with the suppression of the Stat1 pathway (13). Moreover, studies in lupus mice and in lupus patients have shown that activation of Stat1 is strongly associated with SLE pathogenesis (14, 15). Given those considerations, that is, that the deficiency of IL-17 in IL-17−/− mice resulted in a modulated activity
of Stat1 (13), we explored the possibility that changes in Stat1 activity could be associated with the protection from SLE in IL-17−/− mice. To this aim, we analyzed immune phenotypes in IL-17−/−/Stat1−/− double-knockout mice. No significant differences were observed in the frequency of CD4, CD8, CD4+CD25+Foxp3+ regulatory T cells (Tregs) and DN T cells in IL-17−/−/Stat1−/− mice that had been treated with pristane and controls that had been treated with saline (Fig. 3). Also, no differences were observed in serum titers of total IgG, anti-ssDNA, anti-RNP, and anti-chromatin autoantibodies between IL-17−/− and IL-17−/−/Stat1−/− mice treated with pristane and saline-treated controls (Fig. 4). Thus, the protection from SLE that is associated with IL-17 deficiency does not depend on Stat1 activity.

**Protection of IL-17−/− mice from SLE is associated with a reduced frequency of DN T cells and an increased frequency of Tregs**

Th17 cell development and effector functions are in a reciprocal relation with those of Tregs, and a fine balance between Th17 and Tregs is crucial for the maintenance of immune homeostasis (16). Therefore, we compared the frequency of CD4+ Tregs between IL-17−/− mice and WT controls. Pristane-treated IL-17−/− mice had an increased frequency of CD4+ Tregs (Fig. 5), suggesting that the lack of IL-17 favored an expansion of CD4+ Tregs. No significant changes were found in the frequency of CD3+CD8+CD28− cells or CD8+CD25− regulatory CD8 T cells between IL-17−/− and WT mice after treatment with pristane (7.2 ± 3.6 versus 8.4 ± 4.2 CD3+CD8+CD28− and 3.9 ± 0.8 versus 3.2 ± 1.2 CD8+CD25− T cells in IL-17−/− mice versus WT mice, respectively, 20 wk after treatment; p values were not significant in both cases). Also, no significant differences in frequency of total CD4+ and CD8+ T cells were observed between IL-17−/− and WT mice, including after treatment with pristane (Fig. 5).

The frequency of DN T cells was evaluated next, because IL-17 is also produced by DN T cells, and DN T cells are expanded in SLE (2, 6). It was found that the deficiency of IL-17 was associated with a reduced frequency of DN T cells in IL-17−/− mice, but not in WT controls (Fig. 5).

**Discussion**

This study provides direct evidence of a promoting role for IL-17 in the pathogenesis of SLE. Although the primary physiologic role of IL-17 is to fight extracellular pathogens, increasing evidence has suggested that IL-17 can have a significant influence in the pathogenesis of several autoimmune diseases (1–4). In SLE patients, IL-17 serum levels were increased as compared with healthy control subjects (3, 17, 18). Moreover, MRL(lpr/lpr) and B6(nRIP−/−) lupus mice had high levels of IL-17 (2, 5, 19), and IL-17 could be detected in injured kidney tissues of SLE patients (6, 19). Additional data indicated that mice lacking the IL-17R had reduced humoral responses (20), and that mice lacking IL-17 were largely protected from the development of glomerulonephritis (21). Although those data underscored the proinflammatory properties of IL-17 in SLE, our findings complement and expand those observations by showing that IL-17 can directly support the pathogenic events that lead to autoantibody production and kidney inflammation in SLE. In this context, we observed an IL-17 dependency of anti-ssDNA, anti-nRNP, and anti-chromatin autoantibodies (as IL-17−/− mice did not develop those autoantibodies), but not of anti-dsDNA Ab. These results suggested that only the production of selected patterns of autoantibodies could depend on IL-17, a finding that is consistent with the lack of a relationship between levels of IL-17 and anti-dsDNA Ab that has been reported by others (18), and with the possibility that IL-17 might have an influence on the timing of autoantibody responses in SLE. This aspect could be interesting because, in SLE, the levels of some autoantibodies (such as anti-RNP) are relatively insensitive to disease activity, whereas others (such as anti-dsDNA) are produced transiently (mainly during periods of disease activity) (22, 23).

We also found that IL-17 deficiency is associated with an expansion of Tregs (which have a protective role in SLE) (24) and a reduced frequency of DN T cells (which have a detrimental role in SLE) (2, 6). This is important because in MRL(lpr/lpr) lupus mice, IL-17-producing DN T cells were expanded in peripheral lymphoid organs and infiltrated the kidneys (2), and the current new
observations point to a capacity of IL-17 to directly modulate key immunoregulatory circuits in SLE.

Regarding the source of proinflammatory IL-17 after pristane administration, it appeared to include multiple immune cell populations. Indeed, in treated animals, there was a >10-fold expansion of both macrophages and neutrophils producing IL-17 in the peritoneum, in addition to a similar expansion of Th17 cells 1 wk after pristane administration (data not shown). Thus, it is likely that multiple IL-17–producing cells can act in concert to promote the proinflammatory events that culminate in the development of lupus manifestations.

In summary, this study attests a causal relation between IL-17 and the development of SLE. This link substantiates the original suggestion to block the IL-17 axis for a therapeutic management of SLE (8). Clinical evaluation of the effects of blocking this cytokine in SLE patients is awaited.

Acknowledgments
We thank Chunlin Cai for technical help.

Disclosures
The authors have no financial conflicts of interest.

References
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