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*J Immunol* 2001; 166:517-521; doi: 10.4049/jimmunol.166.1.517
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Reduced Incidence and Severity of Collagen-Induced Arthritis in Mice Lacking IL-18

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We have recently reported the presence and a potential proinflammatory role of IL-18 in the synovium of patients with rheumatoid arthritis. To obtain direct evidence that IL-18 plays an influential role in articular inflammation, we investigated the development of collagen-induced arthritis in a strain of mice lacking IL-18 (IL-18−/−) of DBA/1 background. IL-18−/− mice developed markedly reduced incidence of arthritis compared with heterozygous or wild-type mice. Of the IL-18−/− mice that developed arthritis, the severity of the disease was significantly reduced compared with the intact mice. This was accompanied by reduced articular inflammation and destruction evident on histology. IL-18−/− mice also had significantly reduced Ag-specific proliferation and proinflammatory cytokine (IFN-γ, TNF-α, IL-6, and IL-12) production by spleen and lymph node cells in response to bovine type II collagen (CII) in vitro compared with wild-type mice, paralleled in vivo by a significant reduction in serum anti-CII IgG2a Ab level. Treatment with rIL-18 completely reversed the disease of the IL-18−/− mice to that of the wild-type mice. These data directly demonstrate a pivotal role of IL-18 in the development of inflammatory arthritis and suggest that antagonists to IL-18 may have therapeutic potential in rheumatic diseases. The Journal of Immunology, 2001, 166: 517–521.
Technologies/BRL)) at 37°C in 5% CO₂. Cells were stimulated with graded concentrations of CII. Proliferation assays were performed in triplicate in U-bottom 96-well plates (Nunc, Roskilde, Denmark) as previously described (5). Supernatants from parallel triplicate cultures were stored at −70°C until estimation of cytokine content by ELISA.

**Cytokine assays**

Murine TNF-α, IFN-γ (R&D Systems), IL-4, IL-6, IL-10, and IL-12 (p40 + p70) (PharMingen, San Diego, CA) were assayed by ELISA using paired Abs according to the manufacturer’s instructions. Lower limits of detection were as follows: IL-4, IL-6, IL-12, and TNF-α all at 10 pg/ml and IL-10 and IFN-γ at 80 pg/ml.

**Anti-collagen Ab ELISA**

Serum anti-collagen II Ab titers were measured by ELISA. Briefly, 96-well plates (Maxisorb; Nunc) were coated with CII (2 μg/ml in 0.1 M NaH₂CO₃) overnight at 4°C, blocked, and serial dilutions of sera were added. Bound IgG1/2a was detected with biotin-conjugated anti-mouse IgG1 or IgG2a (PharMingen), respectively, and developed as previously described (5). Plates were read at 630 nm.

**Statistical analysis**

This was performed using Minitab software for Macintosh (Cupertino, CA). Clinical and histological scores were analyzed with the nonparametric Mann-Whitney U test. Differences between cumulative incidences at a given time point were analyzed by the χ² contingency analysis. Cytokine and collagen-specific IgG levels were compared using Student’s t test. Bonferroni’s correction for multiple comparison was applied to Fig. 1.

**Results and Discussion**

To compare the development of CIA in IL-18⁻/- and their heterozygous and wild-type littersmates, mice were immunized with CII in CFA on day 0 and boosted with CII in PBS on day 21. Wild-type (n = 34) and heterozygous (n = 21) mice developed inflammatory arthritic disease indistinguishable from each other. Joint swelling was evident from day 25 and up to 90% of mice developed arthritis (Fig. 1). In contrast, IL-18⁻/- mice (n = 30) developed markedly reduced incidence of articular disease compared with wild-type mice (Fig. 1A). Of the IL-18⁻/- mice that developed articular inflammation, the severity of the disease was significantly reduced (p < 0.01) compared with involved wild-type mice (Fig. 1B). This reduced severity in the IL-18⁻/- mice was sustained throughout the chronic phase of arthritis. These results demonstrate that IL-18 is not only required for the initiation of the articular disease, it also sustains the inflammatory process characteristic of CIA.

To determine whether the lack of IL-18 altered erosive arthritic disease, histological examination of the hind limb joints was performed on day 37. As expected, the arthritic wild-type mice revealed extensive mononuclear and polymorphonuclear infiltration of the synovial membrane with synovial hyperplasia and adjacent cartilage and bone erosion. In contrast, the mutant mice show only a mild disease which was markedly less severe compared with that of the wild-type mice (Fig. 2).

We next investigated immunological parameters subserving different pathological response. To compare the expression of cytokines in serum, arthritic mice were selected from homozygous and wild-type mice during the acute phase (day 37) of the disease. IL-4 and IL-5 were not detected. In contrast, high levels of IFN-γ, TNF-α, and IL-6 were detected in wild-type mice. These were present as significantly reduced concentrations in the IL-18⁻/- mice (Table I). IL-12 (p40/p70) was not detected whereas IL-10 was present only at low levels and was comparable in both groups (Table I). We also determined anti-collagen Ab production. Collagen-specific IgG2a Abs, which are typically produced during Th1 response, were significantly lower (p < 0.02) in the IL-18⁻/-
mice compared with wild-type mice while IgG1 Abs remained at similar levels (Fig. 3).

We next sought further evidence for an altered immune response in mice lacking IL-18 by culturing spleen and draining lymph node cells from IL-18−/− and wild-type mice with equivalent articular indices at day 37 or day 55. Cells were cultured with graded concentrations of CII and T cell proliferation and cytokine production were determined. Cells from arthritic wild-type mice proliferated vigorously in response to CII in a dose-dependent manner. This response was significantly reduced in the IL-18−/− mice (Fig. 4). Cells from the arthritic IL-18−/− mice also produced markedly less IFN-γ, TNF-α, IL-6, and IL-12 compared with similarly treated cells from the arthritic wild-type mice in both the acute and the chronic phases of the disease (Fig. 4). IL-4 and IL-10 were not detectable in the culture supernatant of both groups of mice. Together, these results clearly demonstrate that IL-18 is required for the induction of the optimal production of proinflammatory cytokines during arthritic disease.

We next investigated whether the reduced articular inflammation in IL-18−/− mice compared with wild-type mice could be reversed by the administration of recombinant IL-18. Mice were primed and boosted with CII as above. IL-18−/− mice were then injected i.p. with 100 ng/mouse/day of murine rIL-18 for 8 days starting day 29, a dose which was previously shown to serve as an adjuvant to induced collagen arthritis in male DBA/1 mice (5). As expected, IL-18−/− mice developed significantly less severe disease compared with wild-type mice. However, this was completely reversed in both incidence and severity by the administration of rIL-18 (Fig. 5). Furthermore, IL-18−/− mice replenished with IL-18 had a cytokine profile (by day 37) indistinguishable from that of the wild-type mice (data not shown).

Using IL-18−/− mice, a critical role of IL-18 in infectious diseases has been identified (13, 15). However, the role of IL-18 in autoimmune disease has yet to be clarified. Elevated levels of IL-18 message and protein have been identified in several inflammatory diseases, including inflammatory bowel disease (16, 17) and RA (4). In the animal model of diabetes (nonobese diabetic mice), IL-18 gene expression was up-regulated (18). However, administration of rIL-18 inhibited diabetes development presumably by counterregulation of Th1-mediated destructive insulitis (19).

### Table I. IL-18−/− have reduced levels of proinflammatory cytokines in serum

<table>
<thead>
<tr>
<th>Cytokine (pg/ml)</th>
<th>Wild Type</th>
<th>IL-18−/−</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>336.7 ± 64.6</td>
<td>121.1 ± 18.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TNF-α</td>
<td>605.3 ± 173.1</td>
<td>200.1 ± 29.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL-6</td>
<td>427.1 ± 53.4</td>
<td>193.5 ± 51.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL-10</td>
<td>29.6 ± 5.1</td>
<td>27.7 ± 8.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Serum cytokine concentrations in wild type and IL-18−/−. Sera were obtained from mice on day 37 and cytokine levels were measured by ELISA. Results are the means ± SD (six mice per group). p < 0.01 (wild-type vs IL-18−/−). Student’s t test.
We now provide here direct evidence that mice lacking IL-18 developed markedly reduced incidence and severity of CIA.

CIA represents an ideal system to explore the diverse inflammatory effects of IL-18 in an inducible autoimmune model. CIA has proven a useful surrogate in which to investigate the role of cytokines in RA. Th1 responses are generally implicated in the pathogenesis of RA (20). Furthermore, proinflammatory cytokines including IL-12, IL-6, TNF-α, and IFN-γ play a pathogenic role in CIA (21–24). However, the role of IL-12 and IFN-γ is complex. The levels of IL-12 and IFN-γ induced during CIA are variable and their effects on CIA development are dependent on the time of administration (21, 23). Our data clearly demonstrate that IL-18 is required for the optimal induction of Th1 cells and proinflammatory cytokines. Although we have not measured the level of IL-18 in mice with CIA, we have previously shown that IL-18R is expressed at a given time point were analysis by the \( \chi^2 \) contingency analysis and the mean articular index was analyzed with the Mann-Whitney \( U \) test. * \( p < 0.05 \). Data are means ± SEM (\( n = 12 \)).

![FIGURE 5. The reduced incidence (A) and severity (B) of arthritis in IL-18\(^{-/-}\) mice was completely reversed to that of the wild-type mice by treatment with rIL-18. IL-18\(^{-/-}\) and wild-type mice were immunized and boosted with CII as described in Materials and Methods. Some of the IL-18\(^{-/-}\) mice were injected i.p. with 100 ng/mouse/day of IL-18 for 8 days, starting on day 29 (arrows). Differences between cumulative incidences at a given time point were analysis by the \( \chi^2 \) contingency analysis and the mean articular index was analyzed with the Mann-Whitney \( U \) test. * \( p < 0.05 \). Data are means ± SEM (\( n = 12 \)).](http://www.jimmunol.org/)

**References**


