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Mechanisms of Inhibition of Collagen-Induced Arthritis by Murine IL-18 Binding Protein

Nirmal K. Banda,* Andrea Vondracek,* Damian Kraus,* Charles A. Dinarello,* Soo-Hyun Kim,* Alison Bendele,† Giorgio Senaldi,‡ and William P. Arend‡*

IL-18 is an important cytokine in autoimmune and inflammatory diseases through the induction of IFN-γ, TNF-α, and IL-1. We report herein that collagen-induced arthritis (CIA) in mice is inhibited by treatment with murine IL-18 binding protein (mIL-18BP). CIA was induced in DBA/1J mice by the injection of bovine type II collagen (CII) in IFA with added Mycobacterium tuberculosis on days 0 and 21. The mice were then treated for 3 wk with PBS or with two doses of mIL-18BP (0.5 and 3 mg/kg) as a fusion protein with the Fc portion of murine IgG1. Both the clinical disease activity scores and the histological scores of joint damage were reduced 50% in mice treated with either dose of mIL-18BP. Proliferation of CII-stimulated spleen and lymph node cells as well as the change in serum levels of IgG1 and IgG2a Ab to collagen between days 21 and 42 were decreased in mice treated with mIL-18BP. The production of IFN-γ, TNF-α, and IL-1β in cultured spleen cells was reduced by in vivo treatment with low dose, but not high dose, mIL-18BP. FACS analysis showed a slight decrease in NK cells and an increase in CD4+ T cells in spleens of mice treated with mIL-18BP. The steady state mRNA levels of IFN-γ, TNF-α, and IL-1β in isolated joints were all decreased in mice treated with both doses of mIL-18BP. The mechanisms of mIL-18BP inhibition of CIA include reductions in cell-mediated and humoral immunity to collagen as well as decreases in production of proinflammatory cytokines in the spleen and joints. The Journal of Immunology, 2003, 170: 2100–2105.

Interleukin-18 is an important regulator of both innate and adaptive immune responses, as well as of nonimmune mechanisms of host defense and inflammation (1). IL-18 was originally described as a mediator of innate immune responses through the induction of IFN-γ production in NK and Th1 cells in cooperation with other cytokines, such as IL-12 (1). IL-18 is further involved in inflammatory diseases through directly inducing TNF-α production in human monocytes and, together with IFN-γ, indirectly stimulating IL-1β (2). However, IL-18 directly stimulated the production of TNF-α, IL-1β, and IL-6 in mouse peritoneal macrophages (3). In addition to enhancing Th1 responses, IL-18 alone can also stimulate Th2 responses through the induction of IL-4 and IL-13 production in T cells, NK cells, mast cells, and basophils (4). Thus, the role of IL-18 in autoimmune and inflammatory disease processes is probably complex, involving several cells and mechanisms.

IL-18 appears to play an important role in the pathophysiology of experimental animal models of inflammatory arthritis. The incidence and severity of collagen-induced arthritis (CIA)3 in mice were worsened by the administration of IL-18 during 4 days following the initial and booster injections of collagen type II (CII) on days 0 and 21 (5). These effects of IL-18 were due both to enhanced Th1 responses with increased IFN-γ production and to an IFN-γ-independent direct stimulation of TNF-α production (6). In addition, IL-18 administration to mice with CIA led to enhanced lymph node (LN) responses to collagen and to increased serum levels of both IgG1 and IgG2a anti-collagen Ab (6). Mice rendered genetically deficient in the production of IL-18 exhibited reduced incidence and severity of CIA with decreased cellular and humoral immunity to collagen (7).

In a different animal model, blockade of endogenous IL-18 by the administration of an anti-IL-18 Ab suppressed streptococcal cell wall arthritis in mice to the same extent in both wild-type and IFN-γ-deficient mice (8). The results of these studies in two experimental animal models of inflammatory arthritis indicate an important proinflammatory role for IL-18 that may be, at least in part, independent of IFN-γ.

IL-18 is also involved in rheumatoid arthritis, as IL-18 mRNA and protein were present in rheumatoid synovial tissue at higher levels than in osteoarthritis (5, 9, 10). In addition, IL-18 potentiates the effects of IL-12 on the induction of IFN-γ production in synovial tissues in vitro and on the stimulation of TNF-α production in synovial fluid macrophages (5, 9, 10). Given the known effects of IL-18 on stimulation of the production of proinflammatory cytokines, IL-18 may be one factor responsible for the overproduction of TNF-α and IL-1β in the rheumatoid synovium.

The effects of endogenous IL-18 may be regulated by a specific antagonist, the IL-18 binding protein (IL-18BP) (11). IL-18BP is a 40-kDa molecule encoded by a separate mRNA that exhibits limited homology to the ligand-binding α-chain of the IL-18R. IL-18BP avidly binds IL-18 and functions as a competitive inhibitor of receptor binding of IL-18 in vitro and in vivo. The production of IL-18BP in monocytes and epithelial cells is stimulated in a feedback loop by IFN-γ (12, 13). IL-18BP is present in high concentrations in normal human sera, with a 10-fold increase during sepsis (14). Four human and two murine isoforms of IL-18BP have
been identified, created by alternative mRNA splicing (15). Human IL-18BP (hIL-18BP) isoform a and murine IL-18BP (mIL-18BP) isoform d demonstrate the highest binding affinities and are active across species. In the present study we administered mIL-18BP to mice with CIA and observed a marked inhibition of the severity of disease. Our results also indicate that the possible mechanisms of the beneficial effects of IL-18BP include a reduction in both cellular and humoral immunity to collagen and a decrease in production of the proinflammatory cytokines TNF-α and IL-18.

Materials and Methods

CIA

CIA was induced in 8- to 10-wk-old DBA/1J mice (The Jackson Laboratory, Bar Harbor, ME) by an intradermal injection of bovine CII according to a recently described adaptation of the standard protocol (16). Each mouse received 100-μl injections containing 200 μg of CII and 200 μg of inactivated Mycobacterium tuberculosis (H37Rv: Difco, Detroit, MI) inIFA on days 0 and 21. Mice were treated between days 21 and 42 with one injection of [3H]thymidine was measured. The results were expressed as the mean ± SEM, and \( p < 0.05 \) was considered significant.

Effect of mIL-18BP on clinical disease activity and joint histology

The incidence of development of arthritis was 100% in all groups. Compared with PBS alone, mice treated with either dose of mIL-18BP between days 21 and 42 showed both a delay in onset of CIA as well as a 50% reduction in clinical disease activity score at each time point examined (Fig. 1). Histological analysis of the joints

Cytokine levels in spleen cell supernatants

Spleens obtained on day 42 were also used for determination of in vivo cytokine production. Suspendions of spleen cells (2 × 107 cells/well) were incubated in triplicate for 48 h at 37°C and 5% CO2 in supplemented RPMI medium alone, with 50 μg/ml heat-denatured CII, or with 5 μg/ml LPS. Cytokines in spleen cell supernatants were measured using specific ELISAs as previously described (16). The lower sensitivity limit was 30 pg/ml for the IFN-γ and IL-10 ELISAs, 15 pg/ml for the TNF-α and IL-1β ELISAs, and 150 pg/ml for the IL-1R antagonist (IL-1Ra) ELISA.

Cytokine mRNA levels in joints

Cytokine steady state mRNA levels in knees dissected from mice sacrificed on day 42 were determined by RNase protection assay. Total RNA was extracted from the joints using TRIzol (Life Technologies, Gaithersburg, MD), and the quality of RNA was determined by agarose gel electrophoresis. Two mouse cytokine multiprobe template sets, mCk-2b and mCk-3b (RiboQuin, SD Pharmingen, San Diego, CA), and the MAXScript in vitro transcription kit (Ambion, Austin, TX) were used for the synthesis of [32P]UTP-labeled antisense RNA probes. Twenty micrograms of RNA from each joint was hybridized with the [32P]UTP-labeled RNA probes using the RPAIII kit (Ambion, Austin, TX). The protected probes were separated using 6% polyacrylamide/7 M Tris base, boric acid, EDTA (TBE)/urea gels (1 mm; Invitrogen, Carlsbad, CA). The TBE/urea gel was transferred to chromatography filter paper and vacuum-dried for 30 min at 70°C before exposing it to a phosphorimager screen for 48 h. The gel was visualized using a Storm PhosphorImager (Molecular Dynamics, Sunnyvale, CA) with analysis of digitized data on the density of individual bands. The densities of cytokine mRNA bands were expressed as ratios of mRNA for GAPDH.

Spleen cell populations

The immunophenotype of cells in the spleens of mice sacrificed at 42 days was determined by flow cytometric analysis. The isolated spleen cells were washed three times with PBS containing 2% heat-inactivated FCS, and 1 × 107 cells were incubated for 30 min at 4°C with the following Ab: B cells, rat anti-mouse CD19 (IgG2a, FITC-conjugated; Caltag); NK cells, rat anti-mouse pan-NK (IgM, PE-conjugated; Caltag); macrophages, rat anti-mouse F4/80 (IgG2b, PE-conjugated; Caltag); and CD4 T cells, rat anti-mouse GK1.5 (IgG2a, PE-conjugated; BD Biosciences, Mountain View, CA). Matched isotype IgG controls were used for each Ab (Caltag). The cells were again washed three times with PBS and 2% FCS, fixed in 2% formaldehyde in PBS with 2% FCS, and analyzed on an EPICS XL flow cytometer (BD Biosciences).

Statistical analyses

ANOVA, with tests for multiple comparisons, was used to examine the data on clinical disease activity scores of CIA, T cell proliferation, anticallogen Ab, cytokine levels in spleen cell supernatants, spleen cell populations by FACs, and cytokine mRNA levels in joints. The normality of all data was confirmed by w-statistics, with the differences in variance examined by the Brown and Forsyth test. Histological data were analyzed by Student’s t test. Pearson’s correlation coefficient was calculated for selected comparisons between potentially related datasets. In all cases data were expressed as the mean ± SEM, and \( p < 0.05 \) was considered significant.
FIGURE 1. Clinical disease activity in CIA mice treated with mIL-18BP. DBA/1J mice were immunized with 200 μg of CII in IFA, with 200 μg of added M. tuberculosis on days 0 and 21. The mice were treated for 3 wk with i.p. injections every 3 days of PBS (n = 14) or mIL-18BP isof orm d as a fusion protein with the Fc portion of murine IgG1. Two doses of mIL-18BP were examined: 0.5 (n = 15) and 3 (n = 10) mg/kg injection. The clinical disease activity of the CIA was determined every other day by two trained observers who were blinded to the treatment and to each other, using a three-point scale for each paw. The data are expressed as the clinical disease activity score (mean ± SEM) for each treatment group vs the days after the initial collagen injection.

also indicated that treatment with either dose of mIL-18BP prevented joint damage ~50% compared with the PBS group (Table I). No differences were observed in either clinical disease activity scores or histological scores between mice treated with either dose of mIL-18BP. Significant correlations were observed between the scores or histological scores between mice treated with either dose of mIL-18BP. A significant correlation was observed between the changes in serum levels of both IgG2a and IgG1 anti-collagen Ab during this 3-wk interval were markedly reduced after treatment with either dose of mIL-18BP (Fig. 3). No significant differences in the changes in serum levels of anticollagen Ab during this 3-wk interval were present between the two doses of mIL-18BP.

T cell proliferation
To determine whether treatment with mIL-18BP in vivo affected cell-mediated immunity to collagen, in vitro T cell proliferation in spleen and LN cells was measured. The values for PHA-induced proliferation were similar using LN or spleen cells from each group of treated mice, with SIs ranging from 3–10 (data not shown). However, marked reductions in collagen-induced T cell proliferation were observed in both spleen and LN cells obtained from mice treated with either dose of mIL-18BP (Fig. 2). With both LN and spleen cells, the SIs in the two groups of mice treated with mIL-18BP were reduced nearly to the level observed with the DBA control mice.

Production of anticollagen Abs
To examine whether treatment with mIL-18BP affected humoral immunity to collagen, serum levels of both IgG2a and IgG1 anticollagen Ab were measured on days 21 and 42. Anticollagen Ab were not detected in the sera of nonimmunized DBA control mice; the low levels of Ab present on day 21 of the immunized mice were not different among the three treatment groups. Increased serum levels of anticollagen Ab were observed between days 21 and 42 in the immunized mice treated with PBS alone. However, the changes in serum levels of both isotypes of anticollagen Ab over 3 wk were markedly reduced after treatment with either dose of mIL-18BP (Fig. 3). No significant differences in the changes in serum levels of anticollagen Ab during this 3-wk interval were present between the two doses of mIL-18BP.

Cytokine production by spleen cells
To assess the effects of in vivo treatment with mIL-18BP on in vitro production of cytokines by cells from a central lymphoid organ, the levels of various cytokines were measured in the supernatants of cells prepared from the spleens of mice sacrificed on day 42. Treatment with 0.5 mg/kg mIL-18BP, but not 3 mg/kg mIL-18BP, led to decreased production of IFN-γ, TNF-α, and IL-1β in

Table I. Histological joint damage in CIA

<table>
<thead>
<tr>
<th>Histological Parameter</th>
<th>CIA Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PBS (n = 9)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>Pannus</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Cartilage damage</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>Bone damage</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Overall damage</td>
<td>13.2 ± 0.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Joints were harvested from mice on day 42, 3 wk after the booster injection of CII. Five different parameters of histological change were assessed in five joints from each animal using a score of 0–5/joint as determined by an observer blinded to the treatment. The data represent the mean ± SEM score per joint.

<sup>b</sup> p < 0.02 between PBS and either dose of mIL-18BP, by Student’s t test.
spleen cells cultured for 48 h with LPS, but not with CII, compared with cells cultured in medium alone (Fig. 4, A–C). However, IL-10 production by spleen cells cultured with LPS was not different among the three treatment groups (Fig. 4D). Lastly, production of the anti-inflammatory cytokine IL-1Ra was increased to the same degree in LPS-stimulated spleen cells isolated from DBA controls or from each treatment group (Fig. 4E).

Spleen cell populations

To determine whether the decreases in cytokine production were due to changes in cell populations, the percentages of B cells, CD4+ T cells, NK cells, and macrophages in spleen cells from mice treated with PBS or 0.5 mg/kg mIL-18BP were analyzed by cell cytometry. A small decrease in the percentage of NK cells (p < 0.002) and a small increase in CD4+ T cells (p < 0.0006) were observed after treatment with IL-18BP (Table II). However, the percentages of both B cells and macrophages were not altered. These small changes in cell percentages in the spleen are not sufficient to explain the observed marked decreases in the in vitro production of IFN-γ, TNF-α, and IL-1β by cultured spleen cells after IL-18BP treatment in vivo.

Cytokine mRNA in joints

To determine the effects of treatment with mIL-18BP on the local production of cytokines, steady state levels of cytokine mRNA were measured in isolated joints. The data were expressed as the ratio of cytokine mRNA to GAPDH mRNA. The mRNA levels for IFN-γ, TNF-α, and IL-1β were markedly decreased in the joints of mice treated with either dose of mIL-18BP compared with PBS-treated mice (Table III). The mRNA levels of IL-1Ra, IL-6, IL-18, migration inhibitory factor, TNF-β, leukotriene-β, and TGF-β were not altered by treatment with mIL-18BP.

Discussion

The results of our studies show that administration of mIL-18BP at the time of the booster injection of CII reduces the progressive severity of CIA: both clinical disease activity and histological scores were decreased by 50%. The mechanisms of this effect include reduction in both cellular and humoral immunity to collagen as well as decreases in the production of the proinflammatory cyto-

FIGURE 3. IgG1 and IgG2a anticollagen Ab production. CIA was induced in DBA/1J mice, and serum was collected on day 21, the time of the booster injection of CII, and at sacrifice on day 42 after 3 wk of treatment with PBS (n = 9), 0.5 mg/kg mIL-18BP (n = 10), or 3 mg/kg mIL-18BP (n = 10). Sera were also obtained from three normal DBA/1J mice (control). IgG1 and IgG2a anti-collagen Ab were determined with specific ELISA, and the results were calculated as arbitrary units. The data are expressed as the change in units per milliliter of IgG1 and IgG2a anticollegen Ab over the 3-wk period for control mice and for each treatment group (mean ± SEM). Significance testing of the results for IgG1 anti-collagen Ab showed: PBS vs 0.05 mg/kg mIL-18BP, p < 0.006; and PBS vs 3 mg/kg mIL-18BP, p < 0.01. For IgG2a anti-collagen Ab, the results were: PBS vs 0.5 mg/kg mIL-18BP, p < 0.002; and PBS vs 3 mg/kg mIL-18BP, p < 0.02.

FIGURE 4. Spleen cell production of IFN-γ, TNF-α, IL-1β, IL-10, and IL-1Ra. CIA was induced with CII immunizations on days 0 and 21, and mice were treated for 3 wk with PBS (n = 9), 0.5 mg/kg mIL-18BP (n = 10), or 3 mg/kg mIL-18BP (n = 10). Mice were sacrificed on day 42, and spleens were harvested from each treated mouse as well as from three normal DBA/1J mice (control). Suspensions of spleen cells were stimulated with 50 μg/ml CII, 5 μg/ml LPS, or medium alone. After 48 h, measurement of cytokine levels in the supernatants was performed using specific ELISA. The data are expressed as the mean ± SEM cytokine concentration for control mice and for each treatment group; these results are from the same cell preparation depicted in Fig. 2. A, IFN-γ; B, TNF-α; C, IL-1β; D, IL-10; E, IL-1Ra. The p values for cytokine levels in supernatants from spleen cells from PBS vs mIL-18BP-treated mice were: IFN-γ, p < 0.001; TNF-α, p < 0.0001; and IL-1β, p < 0.002.
Table II. Spleen cell subpopulations

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Mice Treated with PBS</th>
<th>Mice Treated with mIL-18BP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG isotype</td>
<td>Anti-cell Ab</td>
</tr>
<tr>
<td>B cells</td>
<td>1.8 ± 0.1</td>
<td>17.8 ± 1.0</td>
</tr>
<tr>
<td>NK cells</td>
<td>2.0 ± 0.04</td>
<td>5.3 ± 0.3b</td>
</tr>
<tr>
<td>Macrophages</td>
<td>2.3 ± 0.3</td>
<td>20.3 ± 0.4</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>2.2 ± 0.4</td>
<td>12.7 ± 0.9a</td>
</tr>
</tbody>
</table>

*Splenocytes were obtained from mice with CIA 42 days after the first injection of collagen, and mice were treated between days 21 and 42 with PBS (n = 5) or 0.5 mg/kg mIL-18BP (n = 5). The spleen suspensions were incubated for 30 min with Ab specific for murine cell types or an IgG isotype control Ab, then stained for FACS analysis. The data are expressed as the percentage of positive cells (mean ± SEM).

*p < 0.002, by ANOVA.

*p < 0.02, by ANOVA.

Table III. mRNA levels in the joints of mice with CIA treated with mIL-18BP

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>PBS (n = 6)</th>
<th>0.5 mg/kg</th>
<th>3 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mIL-18BP (n = 5)</td>
<td>mIL-18BP (n = 5)</td>
<td>mIL-18BP (n = 5)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>1.06 ± 0.29</td>
<td>0.12 ± 0.02a</td>
<td>0.09 ± 0.03a</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.93 ± 0.14</td>
<td>0.06 ± 0.02a</td>
<td>0.07 ± 0.04a</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.27 ± 0.08</td>
<td>0.004 ± 0.003b</td>
<td>0.08 ± 0.05a</td>
</tr>
<tr>
<td>IL-1Ra</td>
<td>0.34 ± 0.16</td>
<td>0.25 ± 0.18</td>
<td>0.24 ± 0.12</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.23 ± 0.05</td>
<td>0.16 ± 0.10</td>
<td>0.29 ± 0.18</td>
</tr>
<tr>
<td>IL-18</td>
<td>0.24 ± 0.08</td>
<td>0.16 ± 0.11</td>
<td>0.26 ± 0.15</td>
</tr>
<tr>
<td>MIF</td>
<td>1.19 ± 0.29</td>
<td>0.92 ± 0.16</td>
<td>0.67 ± 0.20</td>
</tr>
<tr>
<td>TGF-β</td>
<td>0.23 ± 0.07</td>
<td>0.08 ± 0.04</td>
<td>0.07 ± 0.04</td>
</tr>
<tr>
<td>LT-β</td>
<td>0.24 ± 0.11</td>
<td>0.08 ± 0.03</td>
<td>0.06 ± 0.08</td>
</tr>
<tr>
<td>TGF-β/1</td>
<td>1.11 ± 0.35</td>
<td>0.55 ± 0.09</td>
<td>0.41 ± 0.13</td>
</tr>
<tr>
<td>TGF-β/2</td>
<td>0.61 ± 0.12</td>
<td>0.53 ± 0.11</td>
<td>0.30 ± 0.07</td>
</tr>
</tbody>
</table>

*Joints were isolated from animals with CIA on day 42, and total RNA was extracted. Cytokine steady state mRNA levels were determined by RNase protection assay and are expressed as ratios of the mRNA level for GAPDH. MIF, migration inhibitory factor; LT-β, leukaemine-β.

*p < 0.05 vs mice treated with PBS, by ANOVA.
of patients with active Crohn’s disease and was produced by both endothelial cells and macrophages (22). Whether the endogenous production of IL-18BP occurs in other human inflammatory diseases as a natural anti-inflammatory mechanism has not been reported.

Part of the protective effect of IL-18BP in CIA may be due to a decreased production of IFN-γ, although IFN-γ has been reported to exhibit both suppressing and enhancing effects in CIA. For example, neutralization of endogenous IFN-γ with a mAb suppressed the development of CIA in mice when administered early in the disease, but enhanced the disease when given later (23). Similar to IL-18 (5), administration of IFN-γ transiently increased the severity of CIA (23). Subcutaneous injections of IFN-γ into rat paws increased both the frequency and the severity of CIA; an increase in CD4+ T cells in the joints and a decrease in anticyclogen Ab production were observed after the IFN-γ administration (24). Intramuscular injections of IFN-γ also enhanced the development of CIA in mice, although without altering anticyclogen Ab production (25). However, mice lacking IFN-γ receptors were more susceptible to CIA and exhibited decreased production of IgG2a anticyclogen Ab (26). It was suggested that endogenous IFN-γ counteracts the development of CIA, possibly through enhancing IL-4 production or decreasing IL-2 production (27). These variable and somewhat inconsistent observations on the role of IFN-γ in CIA may be due to differences between exogenous and endogenous IFN-γ at different times in the disease process.

It is highly likely that a major mechanism for the protective effect of mIL-18BP in CIA is mediated through inhibition of TNF-α and IL-1β production. These cytokines are synthesized in the joints early in CIA and are responsible in large part for inducing tissue damage in this experimental animal model of arthritis (28, 29). Anticytokine treatment of various animal models of inflammatory arthritis suggests that TNF-α may be more involved in inflammation, and IL-1 in destruction of cartilage and bone (30). The success of TNF-α and IL-1 blockade in the treatment of rheumatoid arthritis has validated the important roles of these cytokines in the pathophysiology of this disease (31). A decreased production of TNF-α and IL-1β by proteins by spleen cells and reduced steady state levels of the mRNA for these cytokines in the joints of mice with CIA were observed in our studies after treatment with mIL-18BP. Decreases in the production of TNF-α and IL-1β may also represent the primary mechanism of CIA amelioration after anti-C5 treatment (16). Inhibition of IL-18 through administration of IL-18BP may be a potential approach for the treatment of patients with rheumatoid arthritis, possibly in combination with anti-C5 or other newer therapies (32).

In conclusion, mIL-18BP at both 0.5 and 3 mg/kg exhibited ~50% inhibition of both clinical and histological changes in CIA. This inhibition correlated with reduced levels of mRNA for IL-1β, TNF-α, and IFN-γ in the joints. Thus, analysis of local cytokine changes in the joints may be more reflective of the efficacy of this reagent than is analysis of events in a central lymphoid organ such as the spleen.

References

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