Important Role of Interleukin-3 in the Early Phase of Collagen-Induced Arthritis

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Objective. Activation of basophils contributes to memory immune responses and results in exacerbation of collagen-induced arthritis (CIA). We undertook the present study to analyze the production and biologic effects of interleukin-3 (IL-3), a strong activator of basophils, in CIA.

Methods. Arthritis was induced by immunization with type II collagen. Mice were treated with blocking monoclonal antibodies against IL-3 or with recombinant IL-3. Clinical scoring, histologic analysis, fluorescence-activated cell sorter analysis, enzyme-linked immunosorbent assay, and cell culturing were performed to assess disease activity and IL-3 production.

Results. IL-3 was produced in large quantities by collagen-specific CD4⁺ T cells in the spleen and was present in the synovial tissue during onset of arthritis, but was down-regulated in paws with severe inflammation. Blockade of IL-3 during the time of arthritis onset resulted in profound improvement of the disease, with reductions in synovial leukocyte and cytokine levels, peripheral blood basophil levels, and anticollagen antibody titers. Blockade of IL-3 during the late phase of arthritis had no beneficial effect. Administration of recombinant IL-3 during onset of arthritis induced a marked exacerbation of the disease, with increased peripheral blood basophil and plasma IL-6 levels and increased titers of anticollagen antibody. In studies of the regulation of IL-3 expression in CD4⁺ T cells, IL-6 and IL-4 suppressed the release of IL-3 by activated CD4⁺ T cells, whereas lipopolysaccharide and CpG DNA up-regulated IL-3 secretion in activated CD4⁺ T cells by acting on costimulatory cells.

Conclusion. Taken together, the present results demonstrate for the first time that IL-3 has an important role in the early phase of CIA.

Interleukin-3 (IL-3), together with IL-5 and granulocyte–macrophage colony-stimulating factor (GM-CSF), belongs to a family of hematopoietic cytokines with 4 short α-helical bundles. Each of these cytokines binds to a unique α-receptor subunit (e.g., IL-3 receptor α [IL-3Rα] for IL-3). Signal transduction is mediated by a common β-receptor subunit, βc, that is unable to bind any of the cytokines (1). In the mouse, a second β-receptor subunit, β₁₃, that associates exclusively with the IL-3Rα subunit, has been identified (2).

IL-3 is produced mainly by CD4⁺ T cells. However, little is known about the regulation of IL-3 secretion from T cells. IL-3 contributes to growth, differentiation, and survival of CD34⁺ hematopoietic progenitor cells. Although disruption of the IL-3 gene does not affect basal hematopoiesis, it is necessary for supporting increased numbers of basophils and tissue mast cells during parasite infection (3). In vitro, IL-3 promotes the differentiation of basophils and mast cells from bone marrow cells (4–7), and it has been reported that it facilitates and induces histamine and IL-4 release by basophils (8–12). In monocyte/macrophages, IL-3 up-regulates class II major histocompatibility complex expression and enhances lipopolysaccharide (LPS)–induced IL-1 secretion (13,14). Together with IL-4 or

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interferon-β (IFNβ), IL-3 supports differentiation of monocytes into dendritic cells (15,16). Induction of osteoclast-like cells by IL-3 has also been described (17,18).

Little is known about the role of IL-3 in arthritis. In an early study, IL-3 messenger RNA was not detected in the synovium of patients with RA (19), and no effect of IL-3 on cultured fibroblast-like synoviocytes was observed (20). Nevertheless, genetic analysis revealed an association between a single-nucleotide polymorphism in the IL-3 promoter and rheumatoid arthritis (21).

Our interest in analyzing the contribution of IL-3 in arthritis was prompted by our recent observation of marked aggravation of collagen-induced arthritis (CIA) (22). Basophils can be activated not only by crosslinkage of surface IgE, but by other factors, especially IL-3, as well. Basophils release not only IL-4, but also the proarthritogenic cytokine IL-6 (see below). Even at very low concentrations, IL-3 induces pronounced release of IL-6 from murine basophils and prolongs the survival of basophils in culture (see below). Therefore, using the DBA/1 mouse model of CIA, we analyzed the expression of IL-3 in the paws at various stages of the disease, quantified the number of basophils and mast cells in the synovial tissue, and investigated how disease incidence and activity were influenced by blockade or administration of IL-3. In addition, we studied the regulation of IL-3 release from T cells in vitro, to better understand the stage-specific release of IL-3 in arthritis.

MATERIALS AND METHODS

Induction of CIA and treatment of mice. Arthritis was induced in male DBA/1 mice by initial subcutaneous injection of 100–200 μg bovine type II collagen (CII) (C1188; Sigma-Aldrich, Munich, Germany) in complete Freund’s adjuvant at the tail base on day 0, and restimulation by intraperitoneal (IP) injection of 100–200 μg CII without adjuvant on day 21. Clinical arthritis was scored on a 0–4 scale under blinded conditions, as follows: 0 = normal, 1 = swelling in 1 joint, 2 = swelling in >1 joint, 3 = swelling of the entire paw, and 4 = deformity and/or ankylosis. In some experiments, animals received daily IP injections of 35 μg of a blocking anti-IL-3 antibody (clone MP2-8F8; Biozol, Munich, Germany) or purified rat IgG (Sigma-Aldrich) from day 21 through day 36. Mice were killed on day 37. In other experiments, daily IP injections of 50 μg anti-IL-3 antibody or purified rat IgG were started when the arthritis score of an individual mouse was at least 2; treatment was continued for 7 days. In additional experiments, mice were treated from day 20 through day 30 with twice-daily IP injections of 100 ng IL-3 (PeproTech, Rocky Hill, NJ) or phosphate buffered saline (PBS). Animal experiments were performed in accordance with the legal requirements of the government of Bavaria (Az. 55.2-1-54-2531-109-05).

Preparation of synovial tissue and quantification of cytokines and infiltrating cells. Fore and hind paws were removed at the ankle joint, the skin was removed from the inflamed paws, and the remaining tissue was carefully recovered with a scalpel in a volume of 500 μl/1,000 μl PBS. Samples were immediately centrifuged for 10 minutes at 400g. The supernatant was immediately frozen and used for enzyme-linked immunosorbent assay (ELISA) of cytokines. The synovial tissue was digested with type I collagenase (Sigma-Aldrich) for 20 minutes at 37°C to obtain a single-cell suspension and used for fluorescence-activated cell sorter (FACS) analysis.

Histologic analysis. Hind paws were fixed in 3.7% formalin for 24 hours, decalcified with RDO rapid decalifier (Medite, Burgdorf, Germany), and embedded in paraffin. At least 10 sections of the tarsometatarsal joints (5 μm thick) were stained with hematoxylin and eosin and scored in a blinded manner on a scale of 0 (normal)–2, for the following categories: synovial inflammation (1 = focal inflammatory infiltrates; 2 = inflammatory infiltrate dominating the cellular histology), synovial hyperplasia (1 = continuous, at least 3-layer-thick synovial lining in 1 joint; 2 = same findings in several joints), pannus formation and cartilage loss (1 = cartilage partially covered by pannus, no cartilage loss; 2 = same findings but with cartilage loss), and bone destruction (1 = small areas of bone destruction; 2 = widespread bone destruction).

Flow cytometry and ELISA for cytokines. The following antibodies were used for flow cytometry or magnetic cell separation: fluorescein isothiocyanate (FITC)–conjugated anti-CD45 (30-F11), allophycocyanin (APC)–conjugated anti-CD4, FITC-conjugated anti-CD11b (M1/70), phyceroerythrin (PE)–conjugated anti-CD11b, Fc block (2.4G2), PE-conjugated anti-CD19 (1D3), APC-conjugated anti–GR-1 (RB6-8C5), APC-conjugated anti-CD4 (RM4-5), PE-conjugated anti–c-Kit (2B8), FITC-conjugated anti-IgE (R35-72), PE-conjugated anti-IL-3 (MP2-8F8), and FITC- and PE-conjugated isotype controls (all from BD Biosciences, San Jose, CA), and APC-conjugated anti-CD49b (DX; Miltenyi Biotech, Bergisch Gladbach, Germany). Unfixed cells were preincubated for 15 minutes on ice with Fc block (5 μg/ml) and then for 45 minutes on ice with combinations of directly labeled antibodies. After 3 washing steps, red blood cells were lysed with FACS lysing solution (BD Biosciences) and samples were analyzed on a FACSCalibur (BD Biosciences). Monocytes and neutrophils were identified by light scatter properties as well as expression levels of CD11b and GR-1. In synovial tissue, mast cells and basophils were identified by expression of IgE and the presence or absence of c-Kit, respectively. In peripheral blood, basophils could be identified by expression of IgE only, because no mast cells are present in the peripheral blood. For quantification of intracellular IL-3, cells were first stained with APC-conjugated anti-CD4 and then treated with Fix-Perm and Perm-Wash solutions according to the instructions of the manufacturer (BD Biosciences) and stained with PE-conjugated antibody against IL-3.

Levels of IL-3, IL-4, and IL-6 were measured with ELISA kits from BD Biosciences. IL-1α, IFNγ, tumor necrosis factor α (TNFα), GM-CSF, and IL-17 were measured with Quantikine ELISA kits (R&D Systems, Wiesbaden, Ger-
many). Antibodies against collagen were quantified by ELISA. Collagen (20 µg/ml) was coated overnight on ELISA plates. Plasma samples were diluted in PBS–3% bovine serum albumin. Immunoglobulins bound to collagen were detected with a horseradish peroxidase (HRP)-labeled polyclonal rabbit antimouse antibody (P260; Dako, Glostrup, Denmark) or HRP-labeled monoclonal antibodies specific for murine IgG1 (clone LO-MG1-2; Serotec, Wiesbaden, Germany) or for murine IgG2a (clone R19-15; BD PharMingen, Heidelberg, Germany). The murine cytokines IL-3, IL-4, and IL-6 were obtained from PeproTech.

Isolation and culture of cells. Splenocytes from mice with CIA were depleted of B cells and CD4+ T cells with magnetic beads directed against CD19 and CD4 (Miltenyi Biotec). Basophils, monocytes, or neutrophils were depleted by incubation of splenocytes with fluorochrome-labeled antibodies against IgE, CD11b, or GR-1 and subsequent incubation of splenocytes with fluorochrome-labeled antibodies against IgG2a (clone R19-15; BD PharMingen, Heidelberg, Germany). Basophils, monocytes, or neutrophils were depleted by magnetic beads directed against CD19 and CD4 (Miltenyi Biotec). Basophils were identified by low expression of CD45 and high expression of DX-5 and constitutive expression of CD45 and DX-5 in combination with propidium iodide (10 µg/ml) and counting beads (Coulter, Krefeld, Germany).

Statistical analysis. Mean ± SEM values were calculated. The significance of group differences was determined by Student’s 1-sided t-test. P values less than 0.05 were considered significant.

RESULTS

IL-3 and basophils in CIA. We first investigated whether IL-3 is produced in the spleen and synovial tissue of mice with arthritis. On day 31 after the first immunization with collagen, total splenocytes or splenocytes depleted of specific leukocyte subsets were restimulated with collagen, and collagen-specific release of IL-3 was determined by subtracting the amount of IL-3 release in the absence of collagen (Figure 1A). Total splenocytes and splenocytes depleted of CD19+ cells (B cells), IgE+ cells (basophils), or GR-1+ cells (mainly neutrophils) produced large amounts of IL-3 after stimulation with collagen. In contrast, depletion of CD4+ T cells or CD11b+ cells (mainly monocytes) completely abrogated the collagen-specific release of IL-3 (Figure 1A), indicating that IL-3 production requires the presence of both CD4+ T cells and CD11b+ costimulatory cells. B cells are neither necessary nor sufficient to support IL-3 production by CD4+ T cells, and the increased release of IL-3 in the absence of B cells resulted from a higher number of T cells and monocytes in the assay, since the total number of leukocytes per well was kept constant and B cells constitute >50% of the leukocytes in the spleen.

Cytokine production in hind paw synovial tissue was measured on day 36 after the first immunization with collagen (Figure 1B). For that purpose the paws were dissected at the ankle joint, the skin was removed, and the soft tissue completely recovered in 1 ml PBS. After centrifugation for 10 minutes at 400g, cytokines in the supernatant were measured by ELISA. Paws were classified into 2 groups according to the degree of clinically apparent arthritis, with 14 paws having a score of 0–2 and 12 paws having a score of 3 or 4. As expected, paws with pronounced inflammation had high levels of IL-6 and IL-1β (mean 683 pg/ml and 619 pg/ml, respectively), while IL-6 and IL-1β levels in paws with no or low-grade inflammation were severalfold lower (144 pg/ml and 72 pg/ml, respectively). TNFα was detectable only at very low levels, but was increased in paws with a clinical score of 3 or 4. In contrast, IL-3 was readily detectable in paws with a score of 0–2 (mean 66 pg/ml), but was highly significantly reduced in paws with severe inflammation (14 pg/ml). Synovial tissue IL-3 levels were
negatively correlated with the clinical arthritis score \( r = -0.75 \). Levels of IL-17, GM-CSF, and IFN\(\gamma\) in the synovium did not correlate with the degree of paw inflammation (Figure 1B).

IL-3 is known to induce and facilitate release of histamine and IL-4 from basophils. We found that IL-3 by itself also induces substantial release of IL-6 from murine basophils and markedly prolongs the survival of isolated basophils in culture (Figure 1C). Release of IL-6 was observed with administration of IL-3 at very low concentrations (half-maximal release at \( 0.3 \text{ ng/ml} \) IL-3). IL-3 also induced release of IL-4 from basophils, but the release of IL-4 was \(~3\)-fold lower than the release of IL-6 (data not shown). In the absence of IL-3, only 6% of the basophils survived 4-day culture in medium, while addition of IL-3 increased the survival of basophils to \(~60\%\) (Figure 1C).

Flow cytometry was performed to analyze whether basophils and mast cells are present in the inflamed paws of mice with CIA. Synovial tissue from inflamed paws was digested with collagenase to obtain a single-cell suspension, and cells were stained with antibodies against IgE, c-Kit, and CD45 to identify basophils (IgE\(^+\), c-Kit\(^+\), and CD45\(^-\)) and mast cells (IgE\(^+\), c-Kit\(^-\), and CD45\(^+\)). While basophils were unambiguously detectable in all inflamed paws at a frequency of \(~0.4\%) of total infiltrating CD45\(^+\) leukocytes, only very few mast cells were found (and only in some of the inflamed paws), with a 20-fold lower frequency than that of basophils (Figure 1D). The majority of infiltrating cells were monocytes and neutrophils, which are also known to be responsive to IL-3.

**Functional analysis of IL-3 in CIA.** The presence of IL-3 in early forms of CIA, as well as the presence of cells (e.g., basophils and monocytes) that are able to respond to IL-3 by releasing proarthritogenic cytokines such as IL-6 or IL-1, suggest that IL-3 might be involved in the pathogenesis of arthritis. We therefore investigated whether blockade of IL-3 with a monoclonal antibody improves the incidence and severity of arthritis in mice injected with 200 \(\mu\text{g} \) CII on day 0 and day 21. One group of mice (\( n = 15 \)) received daily IP injections of anti-IL-3 antibody (35 \(\mu\text{g/day} \)) from day 21 through day 36, while the control group (\( n = 15 \)) was injected with rat IgG at the same dose and time intervals. Blockade of IL-3 during the time of disease onset highly significantly reduced the clinical severity of arthritis. On day 37, the mean arthritis score in the control group was 5.3, whereas in the anti–IL-3 treatment group it was 1.9 (Figure 2A). The maximum score of 4 was reached in 3 of 60 paws from mice in the anti–IL-3 group and 14 of 60 from mice in the control group. The incidence of

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**Figure 1.** Levels of interleukin-3 (IL-3) and frequency of basophils in collagen-induced arthritis. A, IL-3 production by splenocytes after restimulation with collagen. On day 31 after mice were first immunized with collagen, total splenocytes or splenocytes depleted of specific leukocyte subsets as indicated were incubated for 3 days with type II collagen. \( ** = P < 0.01 \) versus total cells. B, Measurement of synovial tissue cytokine levels in hind paws with a low amount of inflammation (score 0–2; \( n = 14 \)) and in those with a high amount of inflammation (score 3 or 4; \( n = 12 \)) on day 36 after induction of arthritis. \( * = P < 0.05; \; ** = P < 0.01 \). GM-CSF = granulocyte–macrophage colony-stimulating factor; TNF\(\alpha\) = tumor necrosis factor \(\alpha\); IFN\(\gamma\) = interferon-\(\gamma\); NS = not significant. C, Influence of IL-3 on activation and survival of basophils in 4-day culture with IL-3 at various concentrations. In the absence of IL-3, there was no detectable release of IL-6 (or IL-4 [data not shown]), and rapid cell death of basophils was observed. Cytokine release and survival of basophils were markedly increased with addition of low amounts of IL-3. Values in A–C are the mean and SEM. D, Flow cytometric detection of basophils and mast cells in single-cell suspensions prepared from synovial tissue from inflamed paws. The frequency of basophils (IgE\(^+\), c-Kit\(^-\)) and mast cells (IgE\(^+\), c-Kit\(^+\)) is shown as a percentage of total CD45\(^+\) infiltrating leukocytes.
arthritis was also significantly reduced (by −50%) on day 37 (Figure 2A).

On day 37, we used the fore paws for analysis of cells infiltrating the synovial tissue and for measurement of IL-6 and TNFα in the supernatant of the recovered synovial tissue (500 μl/paw). The hind paws were used for histologic evaluation. The number of monocytes, the number of basophils, and the total number of CD11b+ cells (including monocytes and neutrophils) recovered per fore paw were significantly reduced in mice treated with anti–IL-3, as was the level of IL-6 measured in the recovered synovial tissue (Figure 2B). Histologic analysis of the hind paws showed that the degree of synovial proliferation and bone destruction was highly significantly reduced in anti–IL-3–treated mice. The amount of infiltrating cells was significantly decreased, and there was a trend toward reduced cartilage destruction ($P = 0.06$) in mice treated with anti–IL-3 (Figure 3). Plasma titers of anticollagen antibodies were reduced in anti–IL-3–treated mice on day 37 (Figure 2C); the reduction

![Figure 2. Clinical and cellular effects of IL-3 blockade during onset of collagen-induced arthritis. Mice (n = 15 per group) were treated with daily injections of anti–IL-3 or rat IgG from day 21 through day 36 after the first immunization with collagen. A, Arthritis score and incidence. B, Analysis of synovial tissue from the fore paws on day 37 after the first immunization with collagen. Absolute numbers of monocytes, neutrophils, and basophils (as identified by flow cytometry) and concentrations of IL-6 and TNFα are shown. C, Analysis of plasma titers of collagen-specific total Ig (plasma dilution 1:100,000) and IgG1 (plasma dilution 1:5,000) and of peripheral blood leukocyte subsets on day 37 after the first immunization with collagen. Values are the mean and SEM. * = $P < 0.05$; ** = $P < 0.01$. OD = optical density (see Figure 1 for other definitions).](https://example.com/figure2)

![Figure 3. Histologic effects of IL-3 blockade during onset of collagen-induced arthritis. Mice (n = 15 per group) were treated as described in Figure 2, and histologic changes in the lower tarsometatarsal joints were determined on day 37 after the first immunization with collagen. A, Sections from a mouse treated with anti–IL-3 and a mouse treated with rat IgG (control). Low-grade synovial hyperplasia without cartilage or bone destruction is seen in the specimens from the anti–IL-3–treated mouse, whereas the control specimens exhibit marked bone destruction and mild cartilage damage, with pronounced synovial hyperplasia (hematoxylin and eosin stained; magnification × 40). B, Summary of histologic scores. Synovial hyperplasia (proliferation), leukocyte infiltration, cartilage erosion (cartilage), and bone destruction (bone) were scored on a 0–2 scale. Values are the mean and SEM. * = $P < 0.05$; ** = $P < 0.01$. See Figure 1 for definitions.](https://example.com/figure3)
was significant for the IgG1 subclass ($P = 0.038$), but not for total Ig ($P = 0.054$) or IgG2a ($P = 0.24$). FACS analysis of the peripheral blood on day 37 revealed a mild but significant reduction in the frequency of basophils, without significant alterations in the frequencies of neutrophils and monocytes (Figure 2C).

These findings show that IL-3 plays an important role in the early phase of CIA. In mice treated with antibodies against IL-3 for only a short period of time (until day 30), arthritis severity was significantly reduced at the end of the treatment period but increased thereafter (data not shown), indicating that the effect of IL-3 antibodies is reversible and that treatment should be continued until the initial inflammation induced by immunization with collagen has declined.

We next analyzed whether blockade of IL-3 is able to reduce the progression of already established arthritis. Mice were immunized twice with 200 μg CII and assessed daily for development of arthritis. When the arthritis score in an individual mouse was at least 2, mice were randomly assigned to receive daily intraperitoneal treatment with anti–IL-3 or rat IgG (n = 10 per group) for 7 days. Blockade of IL-3 did not reduce the progression of established arthritis. Values are the mean ± SEM. See Figure 1 for definitions.

Figure 4. Clinical effects of IL-3 blockade after the onset of collagen-induced arthritis. After induction of arthritis, mice were evaluated daily for clinical disease severity. When the arthritis score was at least 2, mice were randomly assigned to receive daily intraperitoneal treatment with anti–IL-3 or rat IgG (n = 10 per group) for 7 days. Blockade of IL-3 did not reduce the progression of established arthritis. Values are the mean ± SEM. See Figure 1 for definitions.

We also investigated whether administration of IL-3 during the period of disease onset could increase the incidence and severity of arthritis. Mice were immunized with 100 μg CII on day 0 and day 21. One group of mice (n = 21) was treated from day 20 through day 30 with twice-daily IP injection of 100 ng IL-3, while the control group (n = 18) was injected with PBS in the same volume. Injection of IL-3 during the period of disease onset significantly increased the incidence and severity of CIA (Figure 5). On day 31 (1 day after the last injection of IL-3), mice treated with IL-3 showed significantly increased plasma titers of anticollagen antibodies, a 2-fold increased frequency of peripheral blood basophils, and almost 5-fold increased plasma levels of IL-6. There was also a slight, but statistically significant, increase in the frequency of basophils in PBS-treated mice from day 19 (1.49% of total leukocytes) to day 24 (1.78% of total leukocytes). Basophilia and increased plasma IL-6 levels were transient: no significant differences between IL-3– and PBS-treated mice were detectable on day 38 (8 days after the last injection of IL-3). These data suggest that the restricted availability of IL-3 limits disease onset and progression in DBA/1 mice immunized with CII and that IL-3 is a disease-accelerating factor in the early phase of arthritis.

Figure 5. Exacerbation of established arthritis by interleukin-3 (IL-3). Mice were treated with twice-daily intraperitoneal injections of 100 ng IL-3 (n = 21) or phosphate buffered saline (PBS; n = 18) from day 20 through day 30 after the first immunization with collagen. A, Arthritis score and incidence. B, Frequency of basophils in the peripheral blood (as identified by flow cytometry), plasma titers of collagen-specific IgG1 (plasma dilution 1:1,000) and IgG2a (plasma dilution 1:2,000), and plasma levels of IL-6 on day 31 after the first immunization with collagen. Values are the mean and SEM. * = $P < 0.05$; ** = $P < 0.01$. OD = optical density.
Regulation of IL-3 production by CD4+ T cells.

Activated CD4+ T cells are considered to be the main cellular source of IL-3. However, there is little information on how IL-3 secretion by T cells is regulated. We therefore investigated which factors up- or down-regulate IL-3 production in vitro. Polyclonal activation of purified CD4+ T cells with a soluble antibody against CD3 and B cells as accessory cells resulted in little production of IL-3. In contrast, when CD11b+ monocytes were used as accessory cells, IL-3 production by CD4+ T cells activated with soluble anti-CD3 was up-regulated >3-fold (Figure 6A). Addition of the TLR ligands LPS and CpG DNA markedly enhanced IL-3 secretion by polyclonally activated CD4+ T cells in the presence of accessory B cells or monocytes (Figure 6A). Stimulation of CD4+ T cells and accessory cells with LPS or CpG DNA in the absence of anti-CD3 did not result in detectable release of IL-3 (data not shown). Activation of CD4+ T cells with a combination of antibodies against CD3 and CD28 immobilized on beads resulted in very high release of IL-3, independent of the presence of accessory cells or stimulation with LPS or CpG DNA (Figure 6A).

To confirm that IL-3 is produced by CD4+ T cells and not B cells or monocytes, we measured intracellular levels of IL-3 by flow cytometry (Figure 6B). CD4+ T cells were cultured for 3 days with anti-CD3 in the presence of LPS-stimulated B cells or LPS-stimulated monocytes. Intracellular staining for IL-3 was detectable only in CD4+ T cells, and not in CD4− B cells or monocytes. Using TLR-4-deficient C3H mice, we analyzed in greater detail how LPS enhances the release of IL-3 (Figure 6C). In experiments using accessory B cells that were unable to respond to LPS, release of IL-3 by CD4+ T cells was not enhanced, and when CD4+ T cells from TLR-4-deficient mice were used, IL-3 production was increased rather than reduced. These data indicate that LPS increases the release of IL-3 by CD4+ T cells by stimulating accessory cells and that the level of costimulation provided to CD4+ T cells critically influences expression of IL-3.

Based on our finding that IL-3 levels were down-regulated in paws with severe arthritis, we explored whether cytokines present at high concentrations in the inflamed joints were able to down-modulate expression of IL-3 by activated CD4+ T cells. CD4+ T cells were activated in the presence of various cytokines (IL-6, IL-4, IL-1β, TNFα, and macrophage inflammatory protein 2 [MIP-2]). Addition of IL-1β, TNFα, or MIP-2 had no effect on the release of IL-3 (data not shown). However, addition of IL-6 or IL-4 significantly reduced the release of IL-3 by activated CD4+ T cells, independent of the costimulatory factors used for T cell activa-
The present findings demonstrate that IL-3 is a very potent activator of basophils and markedly prolongs their survival in vitro, and that administration of IL-3 in vivo results in increased plasma levels of anticollagen antibodies, 2-fold increased numbers of basophils in the peripheral blood, and 5-fold increased plasma IL-6 levels. However, it must be kept in mind that plasma levels of anticollagen antibodies do not correlate very well with severity of arthritis and that IL-3 also has several other target cells (e.g., monocytes and dendritic cells, as noted above) that may contribute to the development of arthritis.

Local effects of IL-3 in the joint may include increasing the release of IL-1 from monocytes and inducing the development of osteoclasts (14,17). In this study we assessed the presence of basophils and mast cells in the inflamed synovial tissue of mice with CIA and found a rather high frequency of basophils (~0.4% of total infiltrating leukocytes), while mast cells were almost undetectable. Due to conflicting results in studies using different models of arthritis and different strains of mast cell–deficient mice (24–26), the role of mast cells in arthritis development is currently unclear. Our data suggest that the proarthritogenic effects of IL-3 may be mediated in part by activation of basophils and are consistent with previous data demonstrating aggravation of arthritis by application of anti-IgE or anti-CCR2 antibodies, both of which are known to activate basophils (22,27).

To better understand why IL-3 expression in the synovial tissue correlates negatively with the severity of arthritis, and how the expression of IL-3 is regulated, we performed in vitro assays with CD4+ T cells, considered to be the main cellular source of IL-3. Apart from results of promoter analysis and findings of suppression by cyclosporin A, available data on the regulation of IL-3 expression by T cells are very limited (28). IL-3 expression has been observed in both Th1 and Th2 cells (29). We showed in this study that production of IL-3 by CD4+ T cells is dependent on the level of co-stimulation provided to the CD4+ T cells. Activation of CD4+ T cells with anti-CD3 in the presence of freshly isolated B cells resulted in little expression of IL-3, whereas the presence of monocytes, or B cells and monocytes, activated with ligands for TLR-4 or TLR-9 markedly up-regulated production of IL-3 by CD4+ T cells. In experiments using intracellular cytokine staining and cells from TLR-4−deficient C3H mice we showed that LPS up-regulated IL-3 production in CD4+ T cells by acting on B cells, but not directly on T cells. It is known that LPS aggravates CIA, whereas blockade of TLR-4 improves it (30,31).
We also analyzed how proinflammatory cytokines present in inflamed joints modulate the secretion of IL-3 by polyclonally activated CD4+ T cells and found that IL-6 and IL-4 down-regulate IL-3 expression by CD4+ T cells, whereas IL-1β, TNFα, and MIP-2 do not. Treatment with a combination of IL-4 and IL-6 almost completely prevented IL-3 production induced by monocytes or LPS-stimulated B cells and monocytes. Since basophil produce large amounts of IL-4 and IL-6, one could postulate that there is negative feedback between activation of basophil and IL-3 production by T cells. Restimulation of total splenocytes and splenocytes depleted of specific leukocyte subsets with CII confirmed that IL-3 is produced almost exclusively by CD4+ T cells and requires the presence of antigen-presenting CD11b+ monocytes. B cells do not support IL-3 production by CD4+ T cells in the absence of monocytes.

In summary, our data demonstrate that IL-3 is involved in the development of CIA. IL-3 may thus represent a novel therapeutic target for early forms of rheumatoid arthritis, or for maintenance therapy for prevention of flares.

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AUTHOR CONTRIBUTIONS

Dr. Mack had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Bruhl, Mack.

Acquisition of data. Bruhl, Cihak, Niedermeier, Denzel, Rodriguez Gomez, Talke, Goebel, Flächy, Stangassinger, Mack.


Statistical analysis. Bruhl, Mack.

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Clinical Images: Gout attack and stiff knee in an airline passenger in economy class

The patient, a 63-year-old man with a history of chronic gout, which was in remission at the time, took a 2-hour plane ride in economy class and developed acute gout in his right knee. One week later, he presented with contracture of the knee and diminished extension. Arthroscopy (A) revealed an easily detachable chalky deposit on the surface of the hyaline cartilage. Adhesions in the suprapatellar pouch, a “starry sky” appearance, and hemorrhagic areas (B) were also observed. These findings suggest that immobility of the knees in a sitting position might lead to clinical flare of gout.

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