ARTHRITIS & RHEUMATISM
Vol. 42, No. 5, May 1999, pp 963–970

HUMAN INTERLEUKIN-17

A T Cell–Derived Proinflammatory Cytokine Produced by the Rheumatoid Synovium

MARTINE CHABAUD, JEAN MARC DURAND, NICOLAS BUCHS, FRANÇOIS FOSSIEZ, GUILLAUME PAGE, LUCIEN FRAPPART, and PIERRE MIOSSEC

Objective. **To investigate the presence and role of interleukin-17 (IL-17) in rheumatoid arthritis (RA), and its regulation by antiinflammatory cytokines.**

Methods. **The production of IL-17 was measured in supernatants of RA, osteoarthritis (OA), and normal synovial tissue pieces cultured ex vivo. Quantification of IL-17 was performed using a specific biologic assay. IL-17 gene expression was investigated by reverse transcriptase–polymerase chain reaction (RT-PCR)-techniques. Immunohistochemistry was used to evaluate the frequency of IL-17–positive cells in synovium. The secretion of IL-17 by synovium was measured in the presence of IL-4, IL-13, and IL-10. In addition, the contributions of exogenous and endogenous IL-17 to IL-6 production by RA synovium were studied.**

Results. **Functional IL-17 was spontaneously produced by 16 of 18 RA (mean ± SEM 41.7 ± 11.4 units/ml), 2 of 12 OA (5.3 ± 4.5 units/ml), and 0 of 3 normal synovial explant cultures. IL-17 messenger RNA expression was demonstrated by RT-PCR in 4 of 5 RA and 0 of 3 OA synovial samples. By immunostaining of RA synovium, IL-17–producing cells were found in the T cell–rich area. Addition of both IL-4 and IL-13 completely inhibited the production of IL-17, whereas IL-10 had no effect. Addition of exogenous IL-17 to RA synovium resulted in an increase in IL-6 production, whereas that of a blocking anti–IL-17 antibody reduced production of IL-6.**

Conclusion. **The T cell cytokine IL-17 was found to be highly produced by RA, but not by OA, synovium. Its production and function were down-regulated by IL-4 and IL-13. These results indicate that IL-17 contributes to the active, proinflammatory pattern that is characteristic of RA. Through the contribution of IL-17, some Th1-like T cells appear to mediate synovial inflammation.**

The synovium in rheumatoid arthritis (RA) is characterized by hyperplasia of synovial lining cells and an intense infiltration by mononuclear cells (1). Interactions between monocyte-macrophage cells and synoviocytes lead to the production of high levels of proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) (2). These cytokines contribute to the chronic inflammatory process, which results in joint destruction (3). Whereas T cells represent a large proportion of the inflammatory cells invading the synovial tissue, T cell–derived cytokines are clearly less abundant in the joint than cytokines produced by the other cell types described above (4). In particular, cytokines such as IL-4 or interferon-γ (IFNγ) have been difficult to detect (5,6).

Human IL-17 is a 20–30-kd glycosylated, homodimeric polypeptide secreted by CD4+ activated memory (CD45+, RO+) T cells (7,8). Although limited in number, studies now suggest that IL-17 may be a soluble factor by which T cells induce or contribute to inflammation. When cultured in the presence of IL-17, fibroblasts were found to sustain CD34+ hematopoietic progenitor cells and direct their maturation toward neutrophils (7), to produce IL-6, IL-8, granulocyte–colony-stimulating factor, and prostaglandin E_2 (7,9).

Supported in part by grants from the European Union (Biomed-2 program contract BMH4-CT96-1698), from the Hospices Civils de Lyon, and from the Association de Recherche sur la Polyarthrite. Dr. Buchs' work was supported by a grant from the Swiss National Science Foundation.

Martine Chabaud, PhD, Jean Marc Durand, MD, Nicolas Buchs, MD, Guillaume Page, Lucien Frappart, MD, PhD, Pierre Miossec, MD, PhD: Hôpital Edouard Herriot, Lyon, France; François Fossiez, PhD: Schering-Plough Laboratory for Immunological Research, Dardilly, France.

Address reprint requests to Pierre Miossec, MD, PhD, Clinical Immunology Unit, Departments of Immunology and Rheumatology, Hôpital Edouard Herriot, 69437 Lyon Cedex 03, France.

Submitted for publication June 22, 1998; accepted in revised form November 16, 1998.

Addition of TNFα to these cultures with IL-17 was found to be synergistic. In addition, IL-17 stimulated the production of IL-1β and TNFα by human macrophages (10). In the context of arthritis, the effects of IL-17 on cartilage were associated with destruction and lack of repair, including activation nitric oxide and catabolic enzymes, with a decrease of chondrocyte proliferation and proteoglycan synthesis (11,12). These results have been confirmed in vivo by intraarticular injection of IL-17 in mice (13). More importantly, IL-17 was recently found to increase the effect of IL-1 and TNFα on synoviocytes, leading to a synergistic enhancement of cytokine production (7,14).

Taken together, these data suggest that IL-17 may be a key activator of T cell–driven inflammation, and thus may contribute to the pathogenesis of RA. Herein, we have assessed the spontaneous production of IL-17 by cultures of synovial membrane pieces from RA patients, and its regulation by the antiinflammatory cytokines IL-4, IL-13, and IL-10.

MATERIALS AND METHODS

Cytokines and reagents. Purified *Escherichia coli* human recombinant IL-4 (10^7 units/mg), IL-10 (10^6 units/mg), IL-13 (9.5×10^6 units/mg), and IL-17 (rIL-17) were from Schering-Plough Research Institute (Kenilworth, NJ). Human recombinant IL-1β (2×10^8 units/mg) was purchased from Sigma (St. Louis, MO).

Synovial samples and synoviocyte cultures. Synovial samples were obtained from 21 patients with RA, diagnosed according to the revised criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) (15), who were undergoing knee or wrist synovectomy or joint replacement. Osteoarthritis (OA) synovial samples were obtained during joint replacement from 12 patients with OA, and normal synovium from 3 patients who were undergoing knee arthroscopy for traumatic ligament lesions. Cultures of synovial membrane pieces were performed as previously described (16). Briefly, fat and fibrous tissues were removed, and synovium was cut into small pieces with a volume of ~5 mm^3. Pieces of synovium were cultured in triplicate in complete medium made of α–modified Eagle's medium with 2 m*M* L-glutamine, 100 units/ml penicillin, 50 μg/ml gentamicin, 20 m*M* HEPES buffer, and 10% fetal calf serum (Gibco, Grand Island, NY). Cultures were performed at 37°C in a 5% CO_2/95% air humidified environment.

To isolate synoviocytes, synovial pieces were finely minced and digested with 4 mg/ml collagenase (Sigma) in phosphate buffered saline (PBS)–Dulbecco's medium (Gibco) for 2–3 hours at 37°C. After centrifugation, cells were resuspended in complete medium, and cultured in 100-mm Petri culture dishes. After 48 hours, nonadherent cells were removed. Adherent cells were cultured in complete medium, and at confluence, were trypsinized and passaged in 150-cm^2 culture flasks. Synoviocytes were used between passages 3 and 8. At this time, they were a homogenous population of fibroblast-like cells, negative for the expression of CD1, CD3, CD19, CD14, and HLA–DR, and positive for the expression of CD10, CD44, and CD54 as determined by fluorescence-activated cell sorter analysis, using fluorescein isothiocyanate–conjugated monoclonal antibodies (mAb) from Becton Dickinson (Mountain View, CA).

Measurement of IL-6 levels. IL-6 levels were measured by 2-site sandwich enzyme-linked immunosorbent assay (ELISA) as previously described (17). Briefly, supernatants or serial dilutions of IL-6 standards (Schering-Plough Research Institute) were incubated for 90 minutes at 37°C in 96-well microtiter plates (Nunc, Roskilde, Denmark), which were coated overnight at 4°C with the mouse 39C3 anti–IL-6 mAb (1 μg/ml), and saturated for 90 minutes at 20°C with PBS–5% bovine serum albumin. After washing, a biotinylated mouse 13A5 anti–IL-6 mAb (1 μg/ml) was added and cultures were incubated for 90 minutes at 20°C. After subsequent incubation with alkaline phosphatase–coupled streptavidin and visualization with orthophenylenediamine (Sigma), the plates were read at 492 nm. Sensitivity of the assay was 50 pg/ml.

Biologic assay for IL-17. Levels of IL-17 in supernatants were measured with a specific bioassay as previously described (14). Synoviocytes (10^4 cells/well) were incubated in 96-well plates in a final volume of 200 μl of complete medium. Samples were preincubated at 37°C for 30 minutes with 1 μg/ml of anti–IL-17 mAb5. This antibody is a mouse IgG1 mAb obtained after immunization with human rIL-17. Incubation with 1 μg/ml of mAb5 was able to completely inhibit the IL-6 production induced by 50 ng/ml of IL-17, whereas the irrelevant mAb MX1 had no effect. This antibody had no effect on the action of IL-1, TNFα, granulocyte–macrophage colony-stimulating factor (GM-CSF), and macrophage colony-stimulating factor (7). Following the preincubation step, IL-17 (50 ng/ml) or 1:2-diluted supernatant (with or without anti–IL-17) was added at the onset of the culture for a 12-hour incubation. Plates were washed before addition of fresh media. Supernatants were collected after 48 hours, and stored at −20°C until cytokine assays. One unit of bioactive IL-17 was defined as the production of IL-6 (expressed in ng/ml) without anti–IL-17 minus the production of IL-6 with anti–IL-17.

Immunohistology. Paraffin-embedded sections of synovial membrane biopsy samples from patients with RA were treated in xylene and dipped in a gradient of ethanol (once in 99% ethanol, once in 95% ethanol, and once in H_2O). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. The sections were then incubated with 5 μg/ml goat polyclonal anti–IL-17 (R&D Systems Europe, London, UK) or normal goat serum with 1% normal human serum. After overnight incubation at 4°C and washing, the sections were incubated with biotinylated mouse anti-goat IgG antibody for 30 minutes at room temperature, followed by streptavidin–peroxidase complex (Dako, Glostrup, Denmark) for 15 minutes and 3,3′-diaminobenzidine tetrahydrochloride (Dako) for 20 minutes. The sections were then counterstained with Mayer's hematoxylin. For double staining with anti–IL-17 and anti-CD3, the previous single staining was followed by inhibition of exogenous peroxidase activity with 3% hydrogen peroxide, then by incubation with a rabbit anti-CD3 polyclonal antibody (Dako), biotinylated anti-rabbit IgG, streptavidin–

peroxidase complex, and finally, 3-amino-9-ethylcarbazole as chromogen.

IL-17 messenger RNA (mRNA) expression. RNA was extracted from synovial biopsy samples by the guanidinium isothiocyanate method (18). To synthesize complementary DNA (cDNA), 1 μg of total RNA was incubated with 2 μg oligodT primer (Boehringer Mannheim, Meylan, France), 0.5 m*M* dNTP, 10 m*M* dithiothreitol, 10 μl 10× reverse transcriptase (RT) buffer, and 20 units RT (Boehringer Mannheim). Polymerase chain reaction (PCR) was performed using 5 μl of this cDNA in the presence of 50 m*M* KCl, 10 m*M* Tris, pH 8.3, 1.5 m*M* $MgCl_2$, 200 μM dNTP, 0.5 μM of each primer, and 2.5 units of *Taq* polymerase (Boehringer Mannheim) in a total of 50 μl, and was incubated at 94°C for 1 minute, 55°C for 1 minute, and 68°C for 1 minute. This cycle was repeated 30 times. Ten microliters of PCR products were separated by electrophoresis in a 1.5% agarose gel, and visualized by ethidium bromide staining. After transfer to a nylon membrane (Boehringer Mannheim) and prehybridization, membranes were hybridized overnight at 68°C with IL-17 and actin cDNA probes labeled with digoxigenin. After stringency washes, membranes were incubated with an antibody conjugated to alkaline phosphatase and chemiluminescence substrate (Boehringer Mannheim). Blots were exposed to radiographic film (Kodak, Rochester, NY). Primer sequences were as follows: IL-17 sense 5′-ATG-ACT-CCT-GGG-AAG-ACC-TCA-TTG-3′, IL-17 anti-sense 5′-TTA-GGC-CAC-ATG-GTG-GAC-AAT-CGG-3′, and actin sense 5′-GGG-TCA-GAA-GGA-TTC-CTA-TGG-3′; and actin anti-sense 5′-CTC-CTT-AAT-GTC-ACG-CAC-GAT-TTC-3′.

Statistical analysis. Results were expressed as the mean ± SEM of the indicated number of experiments. Differences between cytokine-treated and control groups were compared with Student's paired *t*-test.

RESULTS

Spontaneous secretion of IL-17 by RA synovium. Since IL-17 induces the secretion of proinflammatory cytokines such as IL-6 and leukocyte inhibitory factor (LIF) by stromal cells, and that of IL-1 and TNFα by macrophages, we looked at its potential contribution in the pathogenesis of RA. We have previously shown that pieces of whole RA synovial tissue in ex vivo cultures spontaneously produced high amounts of IL-1β, TNFα, and IL-6 (16). Thus, we measured the spontaneous production of IL-17 in the same ex vivo model. As controls, we used samples of OA and normal synovium.

Pieces of synovium were cultured for 7 days and the supernatants were collected. Activity of IL-17 in these supernatants was measured with a specific bioassay. IL-6 production by synoviocytes, which was induced by the presence of IL-17–containing supernatants, was assessed with and without a blocking anti–IL-17 mAb. The results are presented in Figure 1. As a positive control, a concentration of 1 μg/ml of mAb5 was able to completely inhibit IL-6 production in response to 50 ng/ml of rIL-17, but an isotype-matched, irrelevant mAb MX1 had no effect. Sixteen of 18 synovial supernatants from different RA patients induced IL-6 production by synoviocytes (mean ± SEM 74.1 ± 18.7 ng/ml, range 6.2–223.4 ng/ml), and this production was inhibited with a blocking anti–IL-17 antibody (mean ± SEM 76 ± 7%

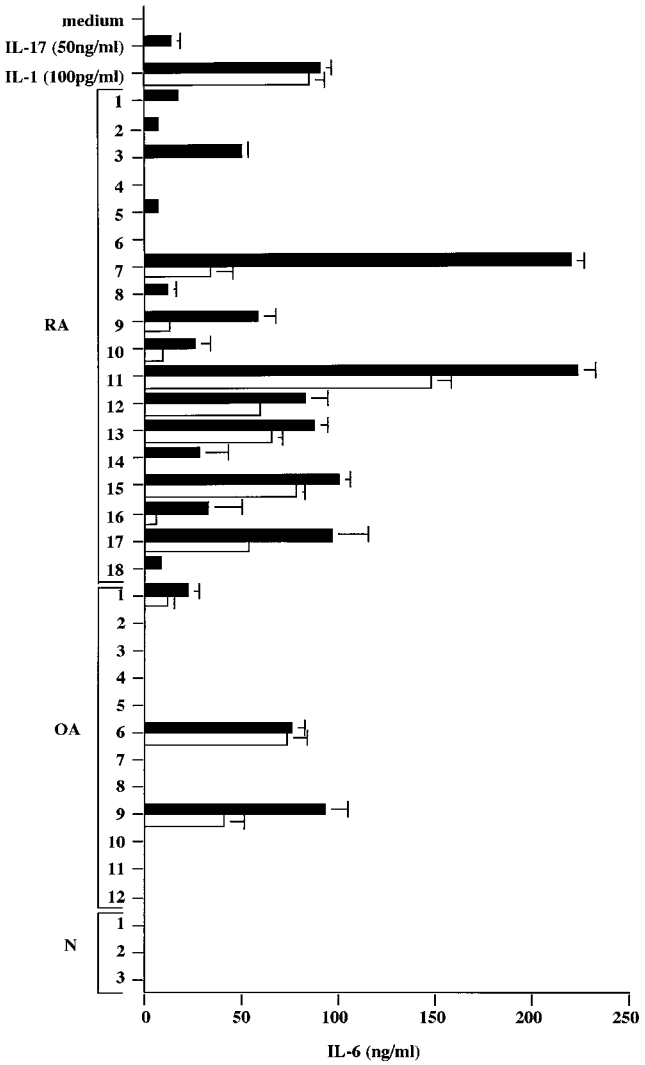

Figure 1. Induction of interleukin-6 (IL-6)-production by IL-17–containing supernatants from synovial membrane pieces. Rheumatoid arthritis (RA), osteoarthritis (OA), and normal (N) synovial pieces were cultured for 7 days in medium alone. Supernatants containing cytokines (IL-1 or IL-17) or synovial pieces were preincubated in the presence (□) or absence (■) of 1 μg/ml of anti–IL-17 (monoclonal antibody 5). The mixture was incubated with synoviocytes for 12 hours. Plates were washed before addition of fresh medium. IL-6 levels were determined by enzyme-linked immunosorbent assay in the 48-hour supernatants. Bars show the mean and SEM.

Table 1. Levels of bioactive interleukin-17 (IL-17) in rheumatoid arthritis (RA), osteoarthritis (OA), and normal (N) synovial sample supernatants*

	IL-17, units/ml
RA-1	16.8
RA-2	6.2
RA-3	49.4
RA-4	0
RA-5	6.8
RA-6	0
RA-7	186.5
RA-8	11.6
RA-9	45.3
RA-10	15.5
RA-11	75.6
RA-12	23.4
RA-13	21.8
RA-14	27.1
RA-15	22.3
RA-16	27.8
RA-17	42.7
RA-18	7.8
OA-1	10.4
OA-2	0
OA-3	0
OA-4	0
OA-5	0
OA-6	0
OA-7	0
OA-8	0
OA-9	53.1
OA-10	0
OA-11	0
OA-12	0
N-1	0
N-2	0
N-3	0

* RA, OA, and normal synovial pieces were cultured for 7 days in medium alone. Cytokines (IL-1 or IL-17) or synovial supernatants were preincubated in the presence or absence of 1 μg/ml of anti–IL-17 (monoclonal antibody 5). The mixture was incubated with synoviocytes for 12 hours. Plates were washed before addition of fresh medium. IL-6 levels were determined by enzyme-linked immunosorbent assay in the 48-hour supernatants. One unit of bioactive IL-17 was defined as the production of IL-6 without anti–IL-17 minus the production of IL-6 with anti–IL-17, expressed in ng/ml.

inhibition). Three of 12 OA synovial supernatants induced IL-6 production (mean ± SEM 63.1 ± 21.6 ng/ml, range 10.4–93.2 ng/ml), and the blocking anti–IL-17 antibody could inhibit the IL-6–inducing activity in 2 of these OA supernatants (mean ± SEM 53 ± 4% inhibition). None of the 3 normal samples induced IL-6 production by synoviocytes.

To better quantify such an effect, 1 arbitrary unit of bioactive IL-17 was defined as the production of IL-6 (in ng/ml) without anti–IL-17 minus the production of IL-6 with anti–IL-17. Accordingly, the mean levels of IL-17 produced by the synovium were 41.7 ± 11.4 units (range 0–186.5) in synovial supernatants, much lower in OA supernatants, as only 2 of 12 were found positive (mean 5.3 ± 4.5 units, range 0–53.1); none of the normal synovial samples showed expression of IL-17 (Table 1).

Expression of IL-17 mRNA in RA synovium. To confirm and extend the previous results, expression of mRNA specific for IL-17 was studied in 5 RA and 3 OA synovial samples using RT-PCR techniques, followed by Southern blot analysis with specific probes. IL-17 mRNA could be detected in 4 of 5 RA samples and in none of the OA samples (Figure 2). Actin was used as an internal control for gene expression.

Presence of IL-17–producing cells in RA synovium. To investigate the presence of IL-17–producing cells, immunostaining of RA synovial sections was performed with an anti–IL-17 polyclonal antibody. As shown in Figure 3, a few cells stained positive for IL-17. Double staining indicated that such cells were localized in the T cell infiltrate, where a significant proportion of the cells stained positive for anti-CD3. IL-17–positive T cells were usually observed at the periphery of the lymphocyte aggregate. An estimation made on several sections from different synovial samples suggested a

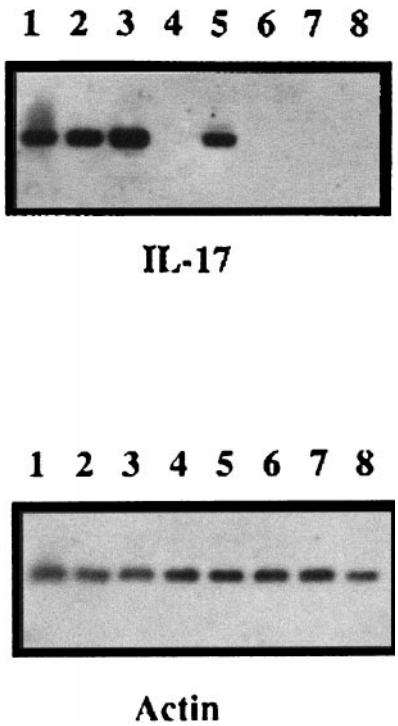

Figure 2. Expression of IL-17 mRNA in synovium from RA and OA patients. Total mRNA was extracted from synovial tissue of 5 RA (lanes 1–5) and 3 OA (lanes 6–8) patients. The results of Southern blot analysis using specific labeled probes are shown for IL-17 (top) and actin (bottom). See Figure 1 for definitions.

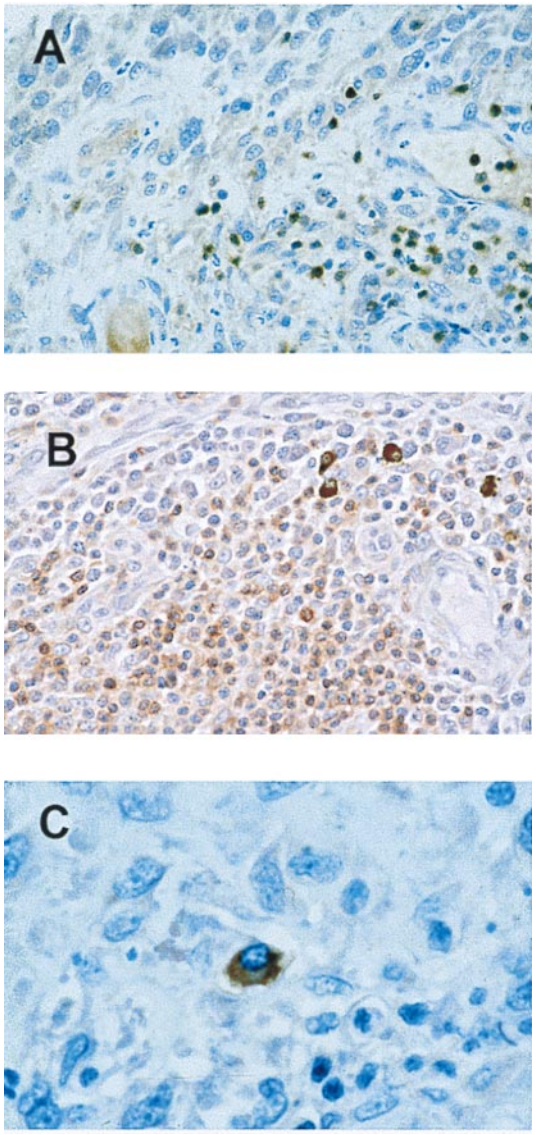

Figure 3. Immunostaining of IL-17–producing cells in RA synovium. Sections of RA synovial tissue were incubated with anti–IL-17 and anti-CD3 antibody and were stained using the immunoperoxidase technique. **A,** Anti–IL-17–positive cells; **B,** double staining with anti–IL-17 (dark brown) and anti-CD3 (red) antibodies; and **C,** close view of IL-17–positive cells localized in the sublining layer. (Original magnification ×400 in **A** and **B,** ×1,000 in **C.**) See Figure 1 for definitions.

frequency of IL-17–producing cells of ~1% of the T cells. No staining of IL-17–positive cells was observed when the anti–IL-17 antibody was first preincubated with human rIL-17 or when normal goat serum was used. Conversely, in normal peripheral blood mononuclear cells stimulated with phytohemagglutinin and ionomycin for 4 hours in the presence of brefeldin, 4% of the cells stained positive for IL-17 (data not shown).

Inhibition of IL-17 production in RA synovium by IL-4 and IL-13. The antiinflammatory cytokines IL-4, IL-13, and IL-10 have been found to inhibit the production of the monocyte-derived cytokines IL-1 and TNFα by RA synovial pieces (16). Using the same assay system, we investigated whether the same effect was observed for the T cell cytokine IL-17. Accordingly, synovial pieces from 5 RA patients were cultured with or without 50 ng/ml of IL-4, IL-10, or IL-13. Such concentrations were previously found to be optimal to inhibit proinflammatory cytokine production by synovial samples (16,19). After 7 days, the supernatants were collected and assayed for IL-17 activity using the bioassay described above. IL-4 and IL-13 completely inhibited the spontaneous production of IL-17 by RA synovium (Figure 4). In contrast, IL-10 had no significant effect on such production, whereas it was able to reduce IL-6 production.

Induction of IL-6 production in RA synovium by IL-17. To extend the proinflammatory properties of IL-17, we investigated whether IL-17 by itself could increase such a proinflammatory pattern. The effects of exogenous IL-17 on IL-6 production by RA synovial

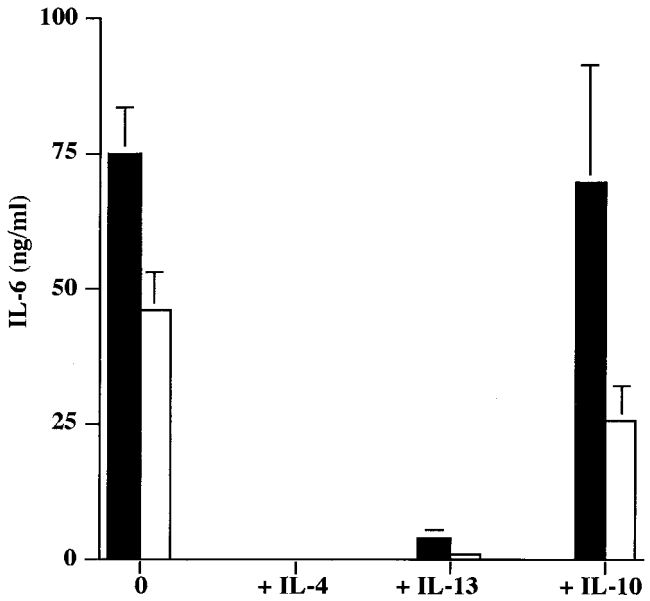

Figure 4. Effect of Th2 cytokines on IL-17 production by RA synovium. RA synovial samples from 5 patients were incubated for 7 days in the presence of IL-4 (50 ng/ml), IL-13 (50 ng/ml), and IL-10 (50 ng/ml). Analysis of IL-17 production was performed using the bioassay described in Figure 1. □ = with anti–IL-17; ■ = without anti–IL-17. See Figure 1 for definitions.

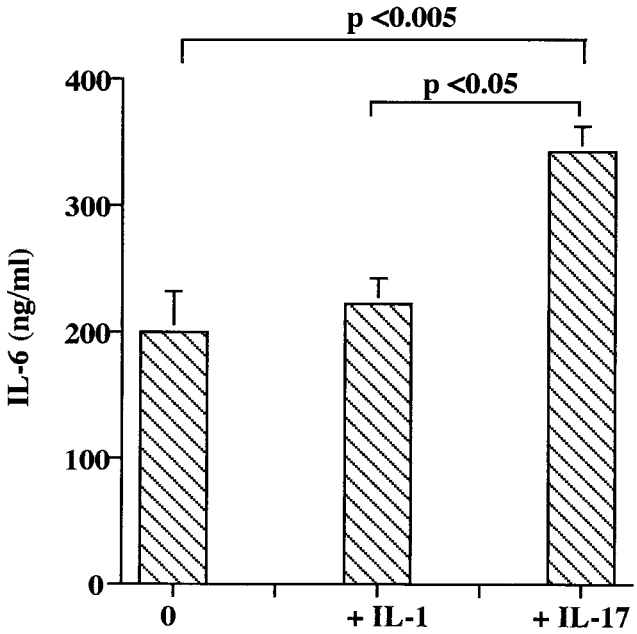

Figure 5. Effect of exogenous IL-17 on IL-6 production by RA synovium. RA synovial samples from 3 patients were incubated for 7 days in the presence of IL-17 (50 ng/ml) or IL-1 (50 ng/ml). Levels of IL-6 in supernatants were measured by enzyme-linked immunosorbent assay. See Figure 1 for definitions.

pieces were analyzed and compared with the effects of IL-1. Samples from 3 patients with RA were cultured in the presence of 50 ng/ml of IL-1 or IL-17, and levels of IL-6 were measured in the supernatants using a specific ELISA. When compared with the spontaneous production of IL-6 (mean ± SEM 199.3 ± 30.7 ng/ml), addition of IL-17 increased such production of IL-6 to an extent that was more potent than that in IL-1 cultures (mean ± SEM 340.3 ± 23.4 ng/ml with IL-17 versus 221.2 ± 26.3 ng/ml with IL-1 [$P < 0.05$]; IL-17 versus control cultures $P < 0.005$) (Figure 5).

Conversely, the bioactivity of endogenous IL-17 was estimated by incubating the samples with the blocking anti–IL-17 antibody mAb5. After 7 days in culture, such blocking resulted in a decrease in IL-6 production by a mean of 54% (range 30–77%) (Table 2). However, this effect was less potent than that of IL-4, which reduced IL-6 levels by a mean of 82% (range 75–90%).

DISCUSSION

It is now established that many proinflammatory cytokines such as TNFα, IL-1, GM-CSF, and chemokines are highly produced by RA synovium, and contribute to joint destruction. Contrasting with such abundance, T cell–derived cytokine proteins have often proven difficult to detect in RA synovium (5). It was thus of interest to evaluate whether this remains a general finding or whether exceptions are still possible. As such, IL-17 is appealing, since it has been described as a T cell–derived cytokine and its properties have been associated with inflammation. Indeed, preliminary results have indicated a significant expression of IL-17 mRNA in inflammatory synovium (20). It was therefore critical to demonstrate whether the protein was present and functional. Thus, we first developed an IL-17–specific bioassay to look at the activity of the secreted protein.

In this study, IL-17 was detected at both the mRNA and protein level in RA synovium, confirming and extending previous preliminary results. IL-17 mRNA was present in 4 of 5 RA and in none of 3 OA samples. Regarding the IL-17 protein, synovial explants from 16 of 18 RA patients produced bioactive IL-17, contrasting with only 2 of 12 OA synovium, which produced much lower levels. In the presence of a blocking anti–IL-17 antibody, the IL-6–inducing activity present in these RA supernatants was reduced by ~70%.

The next question was to investigate the origin of IL-17 in RA synovium. IL-17 has been described as a T cell–specific cytokine. Immunostaining of RA synovium with anti–IL-17 and anti-CD3 confirmed the colocalization of the positive cells. IL-17–positive cells were found at the periphery of the lymphocyte aggregates, which is characteristic of RA synovium. This is consistent with the lower or absent IL-17 production that was observed

Table 2. Effect of anti–IL-17 on IL-6 production by RA synovium*

	IL-6, ng/ml
Experiment 1	
Medium	51.8 ± 4.8
Anti–IL-17	26.9 ± 6.2
Irrelevant mAb	53.4 ± 2.5
IL-4	12.6 ± 3.6
Experiment 2	
Medium	158.9 ± 2.9
Anti–IL-17	114.4 ± 2.5
IL-4	11.6 ± 1.1
Experiment 3	
Medium	30.6 ± 9.9
Anti–IL-17	7.1 ± 5.1
Irrelevant mAb	32.3 ± 4.5

* Values are the mean ± SEM. RA synovial explants were incubated with or without anti–IL-17 (10 μg/ml), irrelevant antibody (10 μg/ml), and IL-4 (50 ng/ml). After 7 days of culture, IL-6 production was measured by enzyme-linked immunosorbent assay. mAb = monoclonal antibody (see Table 1 for other definitions).

in OA and normal synovium, since the T cell infiltrate in these samples was low or absent.

Estimation of the frequency of IL-17–producing cells is obviously difficult. By looking at a number of sections, ~1% of the T cells in RA synovium stained for IL-17. Such a low frequency appears to be consistent with previous results in studies of other T cell–derived cytokines such as IFNγ. In 1 study, 1 of 300 CD3+ T cells in RA synovium expressed IFNγ mRNA, as assessed by in situ hybridization (21). However, other studies using sensitive and specific RT-PCR and ELISA methods were not able to detect IFNγ mRNA and protein (21,22). In contrast, the present study indicates that IL-17 is indeed expressed, secreted, and functional in RA synovium.

Another approach to analyzing the T cell contribution to the pathogenesis of RA has been to establish T cell clones derived from the synovium (23). Such studies have led to the classification of RA as a Th1-like disease (4). Indeed, most clones from RA synovium were found to predominantly produce IFNγ, sometimes in combination with low levels of IL-4 and IL-10. In contrast, T cell clones predominantly producing IL-4 were rarely found. Extension of such studies to IL-17 are now in progress. Preliminary results with RA synovium T cell clones indicate that IL-17 is always produced in association with IFNγ, but not with IL-4 (24). Such findings indicate that IL-17 can be classified as a Th1-like cytokine, consistent with its role in inflammation described herein.

The IL-17 produced spontaneously by RA synovial cultures was shown to play a proinflammatory role in RA. We looked at the production of IL-6, a well-characterized marker of the effect of proinflammatory cytokines such as IL-1 and TNFα (25,26). Blocking IL-17 in RA synovial cultures with a neutralizing mAb resulted in a 20–50% decrease in the production of IL-6. Similar results were observed for LIF production (14). Conversely, addition of IL-17 had an enhancing effect, which was more potent than that of IL-1. Such an effect could be the consequence of the enhancing stimulation of IL-17 on IL-1 and TNFα production by macrophages, as recently demonstrated (10). The interaction of IL-17 with other cytokines within the complex cytokine network is just being documented. Indeed, IL-17 has been shown to potentialize the effect of IL-1 on IL-6 and LIF production by synoviocytes. Moreover, IL-1 and IL-17 have been demonstrated to up-regulate IL-6 production by synoviocytes in a synergistic manner, when used at low concentrations (14). Such an effect could explain the important reduction in IL-6–inducing activity when IL-17 was inhibited. This suggests a critical regulatory contribution of some T cells on monocytes that produce destructive cytokines such as IL-1 and TNFα. In the presence of such a subset of Th1 cells producing IL-17, the action of the monocyte-derived cytokines appears to be further enhanced. Such a signal could be necessary for the destructive effect that is the hallmark of RA.

The T cell–derived cytokine IL-4, which has been shown to inhibit proinflammatory cytokine production by monocytes and RA synovial pieces, was found to also be a strong inhibitor of IL-17 production by cultures of synovial pieces. IL-13, which shares most of its effects with IL-4 (27), was as potent. Both Th2 cytokines were thus able to inhibit the production and the action of IL-17. These findings are consistent with our previous results, which showed that LIF production by synoviocytes, stimulated by IL-17 alone or in combination with IL-1, was also inhibited by IL-4 and IL-13 (14). Not all cytokines classified as antiinflammatory were able to block IL-17 production; in particular, addition of IL-10 had no effect. This finding could be related to the elevated levels of endogenous IL-10 locally produced by synovial monocytes (28,29). In keeping with this, IL-10 was found to have no effect on LIF or IL-6 production by either synovial pieces or isolated synoviocytes (19). The lack of effect of IL-10 on IL-17 production may explain why IL-17, but not IFNγ, was still produced in the synovial explant supernatants. Indeed, production of IL-10 by synovial monocytes appears to be a potent inhibitor of local INFγ production (30).

In conclusion, IL-17 represents a new member of the proinflammatory cytokine family produced by RA synovium and inhibited by some Th2 cytokines. The suppression of both the secretion and action of IL-17 by IL-4 or IL-13 is of potential clinical importance. Reduction of synovial inflammation may be protective through a direct effect on the IL-17–inducing proinflammatory effects, as well as through an indirect effect by reducing the inflammatory/destructive properties of monocyte-derived cytokines. Thus, IL-17 appears to represent a target for treatments of RA, as shown for IL-1 and TNFα.

REFERENCES

1. Harris ED. Rheumatoid arthritis: pathophysiology and implications for therapy. N Engl J Med 1990;322:1277–89.
2. Arend WP, Dayer J-M. Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. Arthritis Rheum 1990;33: 305–15.
3. Lipsky PE, Davis LS, Cush JJ, Oppenheimer-Marks N. The role of cytokines in the pathogenesis of rheumatoid arthritis. Springer Semin Immunopathol 1989;11:123–62.

4. Miossec P, van den Berg W. Th1/Th2 cytokine balance in arthritis. Arthritis Rheum 1997;40:2105–15.
5. Firestein GS, Alvaro-Gracia J, Maki R. Quantitative analysis of cytokine gene expression in rheumatoid arthritis. J Immunol 1990;144:3347–53.
6. Miossec P, Naviliat M, Dupuy d'Angeac A, Sany J, Banchereau J. Low levels of interleukin-4 and high levels of transforming growth factor β in rheumatoid synovitis. Arthritis Rheum 1990;33:1180–7.
7. Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, Maat C, et al. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J Exp Med 1996;183:2593–603.
8. Yao Z, Fanslow WC, Seldin MF, Rousseau AM, Painter SL, Comeau MR, et al. Herpesvirus saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. Immunity 1995; 3:811–21.
9. Yao Z, Painter SL, Fanslow WC, Ulrich D, Macduff BM, Spriggs MK, et al. Human IL-17: a novel cytokine derived from T cells. J Immunol 1995;155:5483–6.
10. Jovanovic DV, DiBattista JA, Martel-Pelletier J, Jolicoeur FC, He Y, Zhang M, et al. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. J Immunol 1998;160:3513–21.
11. Attur MG, Patel RN, Abramson SB, Amin AR. Interleukin-17 up-regulation of nitric oxide production in human osteoarthritis cartilage. Arthritis Rheum 1997;40:1050–3.
12. Lotz M, Bober L, Narula S, Dudler J. IL-17 promotes cartilage degradation [abstract]. Arthritis Rheum 1996;39 Suppl 9:S120.
13. Dudler J, Busso N, Péclat V, Lotz M, So A. In vivo effects of murine recombinant interleukin-17 on synovial joint in mice [abstract]. Arthritis Rheum 1997;40 Suppl 9:S273.
14. Chabaud M, Fossiez F, Taupin JL, Miossec P. IL-17 enhances the effects of IL-1 on IL-6 and LIF production by rheumatoid arthritis synoviocytes. J Immunol 1998;161:409–14.
15. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
16. Miossec P, Briolay J, Dechanet J, Wijdenes J, Martinez-Valdez H, Banchereau J. Inhibition of the production of proinflammatory cytokines and immunoglobulins by interleukin-4 in an ex vivo model of rheumatoid synovitis. Arthritis Rheum 1992;35:874–83.
17. Abrams JS, Roncarolo M-G, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. Immunol Rev 1992;127:5–24.
18. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–9.
19. Dechanet J, Taupin JL, Chomarat P, Rissoan MC, Moreau JF, Banchereau J, et al. Interleukin-4 but not interleukin-10 inhibits the production of leukemia inhibitory factor by rheumatoid synovium and synoviocytes. Eur J Immunol 1994;24:3222–8.
20. Kirkham B, Portek I, Lenarcyzk A, Stavros B, Edmonds J. Interleukin-17 mRNA expression in rheumatoid and psoriatic arthritis synovial membrane [abstract]. Arthritis Rheum 1997;40 Suppl 9:S198.
21. Simon AK, Seipelt E, Sieper J. Divergent T-cell cytokine patterns in inflammatory arthritis. Proc Natl Acad Sci U S A 1994;91: 8562–6.
22. Chen E, Keystone EC, Fish EN. Restricted cytokine expression in rheumatoid arthritis. Arthritis Rheum 1993;36:901–10.
23. Quayle AJ, Chomarat P, Miossec P, Kjeldsen-Kragh J, Førre O, Natvig JB. Rheumatoid inflammatory T-cell clones express mostly Th1 but also Th2 and mixed (Th0-like) cytokine patterns. Scand J Immunol 1993;38:75–82.
24. Aarvak T, Chabaud M, Miossec P, Natvig JB. Analysis of IL-17 and other cytokines and surface markers of RA inflammatory T cell clones [abstract]. Arthritis Rheum 1997;40 Suppl 9:S272.
25. Okamoto H, Yamamura M, Morita Y, Harada S, Makino H, Ota Z. The synovial expression and serum levels of interleukin-6, interleukin-11, leukemia inhibitory factor, and oncostatin M in rheumatoid arthritis. Arthritis Rheum 1997;40:1096–105.
26. Chomarat P, Rissoan MC, Pin JJ, Banchereau J, Miossec P. Contribution of IL-1, CD 14, CD 13 in the increased IL-6 production during monocyte synoviocyte interactions. J Immunol 1995;155:3645–52.
27. De Waal Malefyt R, Figdor CG, Huijbens R, Mohan-Peterson S, Bennet B, Culpepper J, et al. Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. J Immunol 1993;151:6370–81.
28. Katsikis PD, Chu CQ, Brennan FM, Maini RN, Feldmann M. Immunoregulatory role of interleukin 10 in rheumatoid arthritis. J Exp Med 1994;179:1517–27.
29. Chomarat P, Banchereau J, Miossec P. Differential effects of interleukins 10 and 4 on the production of interleukin-6 by blood and synovium monocytes in rheumatoid arthritis. Arthritis Rheum 1995;38:1046–54.
30. Chomarat P, Rissoan M-C, Banchereau J, Miossec P. Interferon γ inhibits interleukin-10 production by monocytes. J Exp Med 1993;177:523–7.