THE JOURNAL OF IMMUNOLOGY

Enhancing Effect of IL-17 on IL-1-Induced IL-6 and Leukemia Inhibitory Factor Production by Rheumatoid Arthritis Synoviocytes and Its Regulation by Th2 Cytokines

Martine Chabaud, François Fossiez, Jean-Luc Taupin and Pierre Miossec

J Immunol 1998; 161:409-414; ;
http://www.jimmunol.org/content/161/1/409

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Enhancing Effect of IL-17 on IL-1-Induced IL-6 and Leukemia Inhibitory Factor Production by Rheumatoid Arthritis Synoviocytes and Its Regulation by Th2 Cytokines[1]

Martine Chabaud,* François Fossiez,† Jean-Luc Taupin,‡ and Pierre Miossec[2]*

IL-17 is a cytokine produced by CD4 T cells that activates the production of inflammatory mediators by synoviocytes. To study the contribution of soluble factors in the interaction between T cells and synoviocytes in rheumatoid arthritis (RA), we looked at the effect of IL-17 on these cells in the presence of cytokines classified as pro (IL-1)- and anti-inflammatory (IL-4, IL-13, IL-10). Both human rIL-1β and rIL-17 induced IL-6 and leukemia inhibitory factor (LIF) production by synovial fibroblasts in a dose-dependent manner. After 7 days of culture, optimal concentrations of IL-1β increased IL-6 (33-fold) and LIF (10-fold) production by synoviocytes, while IL-17 showed a lesser effect on IL-6 (17-fold) and LIF (4-fold) production. Using low concentrations of IL-17 and IL-1β in combination, a synergistic effect was observed on the production of IL-6, whereas an additive effect was observed for LIF production. Production of biologically active IL-17 was demonstrated in RA synovium supernatants with the use of a blocking anti-IL-17 Ab. Both IL-4 and IL-13 had a modest stimulatory effect on IL-1- and IL-17-induced production of IL-6, but inhibited that of LIF. In contrast, IL-10 had a limited inhibitory effect on IL-6 production and no effect on that of LIF. These findings indicate that low levels of cytokines produced by monocytes (IL-1) and T cells (IL-17) can act together on synoviocytes. Thus, some RA synovium T cells producing IL-17 can activate mesenchymal cells leading to an increased proinflammatory pattern sensitive to Th2 cytokine regulation. *The Journal of Immunology*, **1998, 161: 409–414.**

Rheumatoid arthritis (RA)[3] is characterized by the chronic inflammation of the synovium with a hyperplasia of synovial lining cells that interact with blood-derived mononuclear cells (1). Monocyte/macrophage-derived cytokines such as IL-1β and TNF-α appear to play a pivotal role in local activation leading to joint destruction (2). They interact with synoviocytes to produce mediators of inflammation such as PGE_2, degrading enzymes such as collagenase, and other cytokines including granulocyte/macrophage (GM)-CSF, IL-6, and leukemia inhibitory factor (LIF) (3–8).

Whereas T cells represent a large proportion of the inflammatory cells invading the synovial tissue, T cell-derived cytokines are less abundant in the joint than cytokines produced by the other cell types described above (9, 10). In particular, the reduced production of IL-4 may contribute to uncontrolled inflammation (11). A similar conclusion was reached for IL-10 and IL-13 (12). These cytokines, also defined as Th2 cytokines (13), have thus been classified as anti-inflammatory on the basis of their inhibitory effect on the production of IL-1, TNF-α, IL-6, and IL-8 by monocytes and synovium samples (11, 12, 14–17).

Other T cell-derived cytokines such as IFN-γ, defined as Th1 cytokines (13), act in an opposite way, contributing to an inflammatory pattern (17). For newly defined cytokines, such classification is still pending. This is the case for IL-17, characterized recently as the human counterpart of mouse CTLA-8. This CD4 T cell-derived cytokine was found to directly activate fibroblasts and synoviocytes, leading to the production of IL-6, IL-8, PGE_2, and granulocyte-CSF (18). In addition, IL-17 was shown to sustain the maturation CD34-hemopoietic progenitors into neutrophils when cultured with fibroblasts (19). In keeping with its effect during inflammatory conditions, IL-17 was shown to have an enhancing effect on nitric oxide production by cartilage (20).

As this cytokine is produced only by activated T cells, we investigated the consequences of monocyte and T cell interaction on synoviocytes by looking at the effect of the T cell-derived cytokine IL-17 and the monocyte-derived cytokine IL-1 on cytokine production by synoviocytes. This was further studied in the presence of the regulatory cytokines IL-4, IL-13, and IL-10.

Materials and Methods

Cytokines and reagents

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

Synovium samples and synoviocyte cultures

Rheumatoid synovium samples were obtained according to the revised criteria of the American College of Rheumatology (21) from patients with RA who were undergoing knee or wrist synovectomy or joint replacement. Synovium piece cultures were performed as already described (11). Briefly, fat and fibrous tissues were removed, and the synovium was cut into small pieces with a volume of approximately 5 mm^3. Pieces of synovium were cultured in triplicate in complete medium made of α-MEM medium (Life Technologies, Grand Island, NY) with 2 mM L-glutamine,

*Departments of Immunology and Rheumatology, Hôpital Edouard Herriot and Laboratoire d' Immuno-Virologie Unité Mixte de Recherche, Centre National de la Recherche Scientifique (CNRS) 5537, Faculté de Médecine Laennec, Lyon; †Schering-Plough Laboratory for Immunological Research, Dardilly; and ‡CNRS Unité de Recherche Associée (URA) 1456, Université de Bordeaux II, Bordeaux, France

Received for publication December 3, 1997. Accepted for publication February 25, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] These studies have been supported in part by grants from the Hospices Civils de Lyon and from the European Union (Biomed-2 Program, contract BMH4-CT96-1698).

[2] Address correspondence and reprint requests to Dr. P. Miossec, Clinical Immunology Unit, Departments of Immunology and Rheumatology, Hôpital Edouard Herriot, 69437 Lyon Cedex 03, France. E-mail address: miossec@cimac-res.univ-lyon1.fr

[3] Abbreviations used in this paper: RA, rheumatoid arthritis; LIF, leukemia inhibitory factor; GM-CSF, granulocyte/macrophage-CSF; M-CSF, macrophage-CSF.

 0022-1767/98/$02.00

100 U/ml penicillin, 50 mg/ml gentamicin, 20 mM HEPES buffer, and 10% FCS. Cultures were performed at 37°C in a 5% CO_2/95%-air humidified environment.

To isolate synoviocytes, synovium pieces were finely minced and digested with 4 mg/ml collagenase (Worthington, Freehold, NJ) in PBS-DMEM (Life Technologies) for 2 to 3 h at 37°C (7). After centrifugation, cells were suspended in complete medium and cultured in 100-mm culture petri dishes. After 48 h, 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 passage 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 FACS analysis, using FITC-conjugated mAbs from Becton Dickinson (Mountain View, CA).

To obtain culture supernatants, synoviocytes were plated in 96-well dishes at 10^4 cells/well in 200 μl of complete medium. The cytokines to be tested were added at the onset of the culture. Supernatants were collected after 7 days of culture.

Measurement of LIF and IL-6 levels

IL-6 levels were measured by two-site sandwich ELISA as previously described (22). Briefly, supernatants or serial dilutions of IL-6 standards (Schering-Plough Research Institute) were incubated for 60 min at 37°C in 96-well microtiter plates (Nunc, Roskilde, Denmark), coated overnight at 4°C with mouse 39C3 anti-IL-6 mAb (1 μg/ml), and saturated for 90 min at 20°C with PBS 5% BSA. After washing, a biotinylated mouse anti-IL-6 mAb (1 μg/ml) was added and incubated for 90 min at 20°C. After subsequent incubation with peroxidase-coupled streptavidin and revelation with orthophenylenediamine (Sigma), the plates were read at 492 nm.

LIF levels were also measured by a two-site sandwich ELISA using two monoclonal anti-LIF Abs (23). Mouse 1F10 anti-human LIF mAb was used for coating and biotinylated mouse 7D2 anti-human LIF mAb for detection. After subsequent incubation with a streptavidin-peroxidase complex and revelation with OPD, the ODs were measured at 492 nm.

Biologic assay for IL-17

Synoviocytes (10^4 cells/well) were incubated in 96-well plates in a final volume of 200 μl of their respective complete medium. Samples were preincubated at 37°C for 30 min with 1 μg/ml of the anti-IL-17 mAb5 (Schering-Plough Research Institute). mAb5 is a mouse IgG1 obtained after immunization with human rIL-17. 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 Ab had no effect on the action of IL-1, TNF-α, GM-CSF, or M-CSF (19). Following the preincubation step, IL-17 (50 ng/ml) or a 1:10 dilution supernatant (with or without anti-IL-17) was added at the onset of the culture for a 12-h incubation. Plates were washed before addition of fresh medium. Supernatants were collected after 48 h and stored at −20°C until cytokine assays.

Statistical analysis

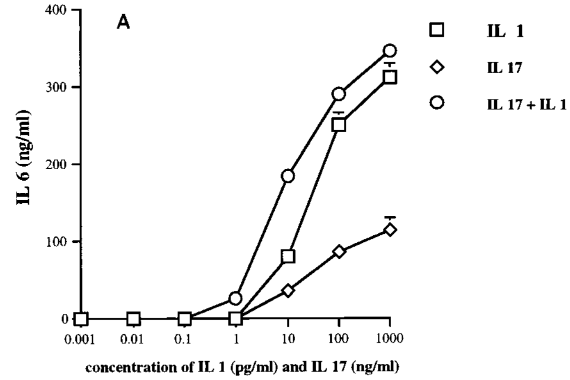
Results were expressed as mean ± SEM of *n* separate experiments. Differences between IL-4- or IL-13- or IL-10-treated groups and the control group were compared with nonparametric Wilcoxon paired *t* test.

Results

Effect of IL-17 on IL-6 production by RA synoviocytes

We investigated whether IL-6 production by synoviocytes could be modulated by the monocyte-derived cytokine IL-1β and by the T cell-derived cytokine IL-17. Synovial fibroblasts were incubated with different concentrations of IL-1 and IL-17. After 7 days of culture, supernatants were collected and assayed for IL-6 production by ELISA. When IL-17 at concentrations ranging from 0.01 to 1000 ng/ml and IL-1 at concentrations ranging from 0.01 to 1000 pg/ml were used alone, IL-6 production was increased in a dose-dependent manner (Fig. 1*A*). IL-1β (100 pg/ml) induced a 33-fold increase of IL-6 production, while IL-17 (10 ng/ml) showed a lesser effect (17-fold increase) (Fig. 1*A*).

When IL-1 and IL-17 were combined, there was an enhancing effect over that seen with IL-1 alone. At high concentrations of IL-17 (>10 ng/ml) and IL-1 (>10 pg/ml), an additive effect was observed until saturation was reached. At lower concentrations of IL-17 (1–10 ng/ml) and IL-1 (1–10 pg/ml) in combination, synoviocytes produced more IL-6 than the sum of IL-6 production by synoviocytes incubated with each cytokine, indicating a synergistic effect. This is further demonstrated in Figure 1*B* where synovial fibroblasts were incubated with combinations of low concentrations of IL-1 (1 and 10 pg/ml) and IL-17 (1, 5, and 10 ng/ml). In particular, synergy was best observed with 1 ng/ml of IL-17 and 1 pg/ml of IL-1, which had no effect on IL-6 production when used alone. In time course studies, this synergistic effect was seen as early as 24 h after culture initiation (data not shown).



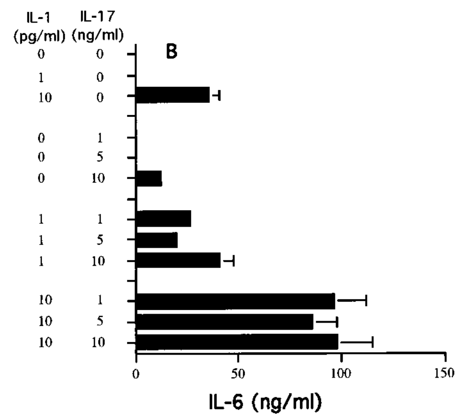


FIGURE 1. Effect of the combination of IL-1β and IL-17 on IL-6 production. *A*, Synoviocytes were cultured with increasing concentrations of IL-1β, measured in pg/ml, and IL-17, ng/ml, used alone and in combination. *B*, Synoviocytes were cultured with the combination of increasing concentrations ranging from 0 to 10 pg/ml of IL-1β and from 0 to 10 ng/ml of IL-17. IL-6 levels were measured by ELISA in day 7 supernatants.

Effect of IL-17 on LIF production by RA synoviocytes

Similarly, we investigated how LIF production by synoviocytes could be modulated by IL-1β and IL-17 (Fig. 2). When IL-17 at concentrations ranging from 0.01 to 1000 ng/ml and IL-1 at concentrations ranging from 0.01 to 1000 pg/ml were used alone, LIF production was increased in a dose-dependent manner (Fig. 2*A*). IL-1β (100 pg/ml) induced a 10-fold increase of LIF production, while IL-17 (10 ng/ml) showed a lesser effect (4-fold increase).

When IL-1 and IL-17 were combined, an additive effect was observed at both low and high concentrations of IL-1 and IL-17 until saturation was reached (Fig. 2*A*). This is further demonstrated in Figure 2*B* where synoviocytes incubated with low concentrations of IL-1 (1–10 pg/ml) and IL-17 (1–10 ng/ml) in combination produced more LIF than the synoviocytes incubated with each cytokine separately, indicating an additive effect. In time course studies, this additive effect was observed as early as 12 to 24 h after initiation of culture (data not shown).

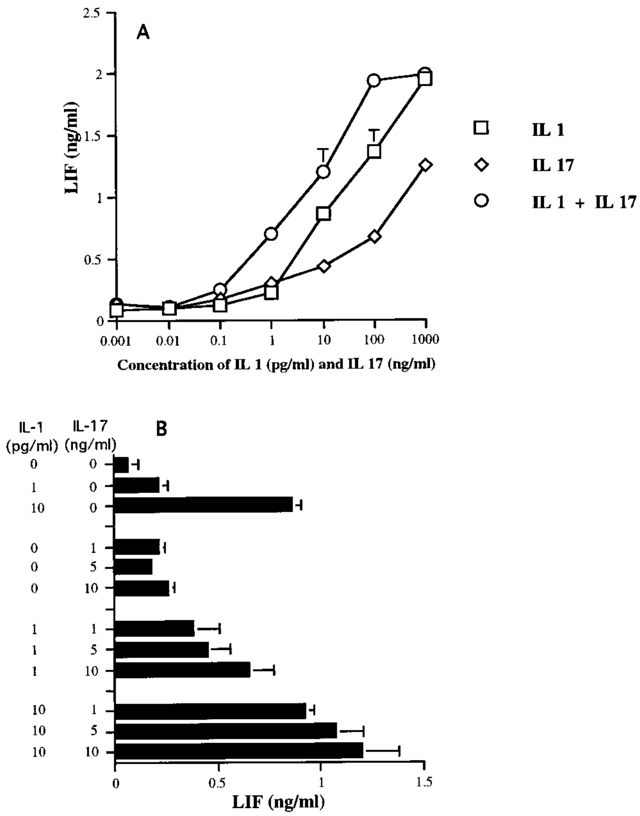


FIGURE 2. Effect of the combination of IL-1β and IL-17 on LIF production. *A*, Synoviocytes were cultured with increasing concentrations of IL-1β, measured in pg/ml, and IL-17, ng/ml, used alone and in combination. *B*, Synoviocytes were cultured with the combination of increasing concentrations ranging from 0 to 10 pg/ml of IL-1β and from 0 to 10 ng/ml of IL-17. LIF levels were measured by ELISA in day 7 supernatants.

Combined together, these results indicate that cytokines produced by monocytes (IL-1) and T cells (IL-17) can act together on synoviocytes at the low concentrations that can be expected to be present in vivo.

Regulation by IL-4, IL-13, and IL-10 of IL-6 and LIF production by RA synoviocytes induced by IL-1 and IL-17

IL-4, IL-13, and IL-10 were tested for their effect on the secretion of IL-6 by synoviocytes stimulated with optimal concentrations of IL-1β and IL-17, alone and in combination. Levels measured after 7 days of culture are presented in Table I. Addition of IL-4 and IL-13 used alone at the onset of culture increased the spontaneous secretion of IL-6 by 4.5-fold. This effect was much lower than that of IL-1 as well as that of IL-17. However, with stimulated cells, a modest 30% enhancing effect of IL-4 and IL-13 was found on IL-6 production by IL-17-, IL-1β-, or IL-1β plus IL-17-stimulated synoviocytes. In contrast, IL-10 had rather a modest inhibitory effect on activated synoviocytes. This effect was less when IL-1β and IL-17 were combined.

As shown in Table II, and as opposed to the effect on IL-6, IL-4 and IL-13 reduced LIF production by unstimulated synoviocytes with a mean inhibition of 60 and 77%, respectively. Similar inhibitory effect was found with IL-1β- or IL-17-stimulated synoviocytes. This effect was less important when IL-1β and IL-17 were both added at the onset of culture. In contrast, IL-10 had no effect on LIF production.

To return to the in vivo situation, similar experiments were performed in the presence of low concentrations of IL-1β (1 pg/ml) and IL-17 (1 ng/ml). These concentrations of IL-17 and IL-1β alone had no effect on IL-6 production, whereas their combination induced the production of 30.3 ng/ml of IL-6, confirming the synergistic effect described above (Fig. 3). Addition of IL-4 and IL-13, but not IL-10, increased the spontaneous secretion of IL-6. The enhancing effect of IL-4 or IL-13 was more important on IL-17 or IL-17 plus IL-1β stimulation. For each of these conditions, IL-4 was in general more potent than IL-13. In contrast, IL-10 had no effect on IL-6 production by synoviocytes, except for an inhibitory effect on IL-17 plus IL-1β-stimulated synoviocytes.

Results obtained for LIF production with low concentrations of IL-1 and IL-17 are shown in Figure 4. Similar inhibitory effects of IL-4 and IL-13 were found with IL-17- or IL-1β-stimulated or unstimulated synoviocytes. This inhibition was less important when the two cytokines were combined. In contrast, IL-10 had no effect on LIF production by stimulated or unstimulated synoviocytes.

IL-6 and LIF induction by IL-17-containing supernatants from RA synovium pieces

It was important to extend these results to natural IL-17 produced by RA synovium. To test its effect on synoviocytes, the biologic activity of IL-17 present in supernatants from RA synovium pieces was measured (Table III). Supernatants collected after 1 wk of culture of RA synovium pieces were preincubated with and without blocking anti-IL-17 mAb before being tested on synoviocytes. As a positive control, the IL-6-inducing effect of rIL-17, but not IL-1, was completely blocked. A 50% reduction of the induction of IL-6 and LIF production by RA synovium supernatants could be specifically blocked by anti-IL-17 mAb 5, but not by an isotype-matched irrelevant mAb, MX1.

Discussion

The RA synovitis is characterized by cell interactions between bone marrow-derived cells such as monocytes, T cells, and B cells

Table I. *Regulation by IL-4, IL-13, and IL-10 of IL-6 production by RA synoviocytes induced by IL-1β and IL-17*[a]

		IL-6 Production (ng/ml)						
Activators	*n*	0	+ IL-4		+ IL-13		+ IL-10	
0	6	9.8 ± 2.8	44.7 ± 6.3*	+356[b]	43.2 ± 12.4*	+340[b]	11.2 ± 1.6**	+14[b]
IL-1	4	496.9 ± 55.1	667.2 ± 67.9***	+34	682.8 ± 93.1***	+37	322.4 ± 61.8***	−35
IL-17	4	282.7 ± 44.2	357.8 ± 61.7***	+27	382.9 ± 55.6***	+35	179.2 ± 62.6***	−37
IL-1 + IL-17	4	597.3 ± 57.8	726.3 ± 39.9***	+22	798.7 ± 80.1***	+34	530.4 ± 47.1***	−11

[a] 10^4 Synoviocytes were incubated in 96-well culture plates for 7 days with IL-1β (100 pg/ml) or IL-17 (20 ng/ml) or their combination in the presence of 50 ng/ml of IL-4, IL-10, or IL-13. After 7 days of culture, IL-6 levels were measured by ELISA and are expressed as the mean ± SEM of *n* separate experiments. Differences in IL-6 production between the IL-4-, IL-13-, or IL-10-treated group and the control group were analyzed with the nonparametric Wilcoxon paired *t* test. $*p < 0.005$; $**p < 0.01$; $***p < 0.0001$.
[b] Column represents % induction.

Table II. *Regulation by IL-4, IL-3, and IL-10 of LIF production by RA synoviocytes induced by IL-1β and IL-17*[a]

Activators	*n*	LIF Production (ng/ml) 0	+ IL-4		+ IL-13		+ IL-10	
0	6	0.22 ± 0.03	0.09 ± 0.02*	−59[b]	0.05 ± 0.02*	−77[b]	0.21 ± 0.04	−4[b]
IL-1	4	1.66 ± 0.26	0.54 ± 0.09**	−67	0.50 ± 0.08**	−70	1.69 ± 0.02	+2
IL-17	4	0.57 ± 0.06	0.19 ± 0.05*	−67	0.13 ± 0.01*	−77	0.53 ± 0.08	−7
IL-1 + IL-17	4	1.92 ± 0.20	0.98 ± 0.07***	−49	0.93 ± 0.14***	−52	1.88 ± 0.31	−2

[a] RA synoviocytes were cultured as described in Table I in the presence of 50 ng/ml of IL-4, IL-10, or IL-13. After 7 days of culture, LIF levels were measured by ELISA and are expressed as the mean ± SEM for *n* separate experiments. Differences in LIF synthesis between IL-4-, IL-13-, or IL-10-treated groups and the control group were analyzed with the nonparametric Wilcoxon paired *t* test $*p < 0.005$; $**p < 0.001$; $***p < 0.05$.

[b] Column represents % inhibition.

and resident mesenchymal cells, namely synoviocytes. The contribution of T cells in the proinflammatory cytokine release has been a matter of debate (24). It is clear that monocyte-derived cytokines such as TNF-α and IL-1 interact with synoviocytes, leading to the production of other cytokines such as IL-6, GM-CSF, and LIF (2). Contrasting with the abundance of monocyte and synoviocyte-derived proinflammatory cytokines, T cell-derived cytokines are difficult to detect in RA synovium (9, 10).

The objective of the present report was to examine the effect of IL-17, a specific T cell-derived cytokine, on synoviocytes in the presence of proinflammatory and anti-inflammatory cytokines. This allowed the study of the contribution of soluble factors in the interaction between T cells and synoviocytes.

IL-17 appears to be a proinflammatory cytokine produced by the RA synovium

IL-17 has been shown to activate the transcription nuclear factor NF-κB and to induce the production/expression of IL-6, IL-8, PGE_2, G-CSF, and ICAM-1 in fibroblasts and the production of nitric oxide by cartilage (18–20). Such effects are shared with proinflammatory cytokines such as TNF-α and IL-1. In the context of inflammation, IL-17 is of interest because it acts directly on various mesenchymal cells including chondrocytes and synoviocytes. Indeed, in RA synovium, T cells are in close contact with monocytes and fibroblasts. Thus it can be expected that factors produced by these cell types act in concert on the resident cells. Indeed, molecules involved in monocyte-synoviocyte interactions leading to enhanced cytokine production have been clarified (25). Here, we show a spontaneous production of biologically active IL-17 by cultures of RA synovium pieces. Results obtained with a blocking anti-IL-17 Ab indicate that IL-17 contributes to ~50% of the IL-6- or LIF-inducing activity present in these supernatants. As a control, supernatants of cultures of osteoarthritis synovium, which contain a reduced T cell infiltrate, did not contain such IL-17-related activity (data not shown).

IL-17 increases IL-1 effect on cytokine production by synoviocytes

The results indicate that the combination of the most potent monocyte-derived cytokine on synoviocytes, namely IL-1, and IL-17 strongly increases their cytokine production. When used alone, both IL-1β and IL-17 induced IL-6 and LIF production by synovial fibroblasts in a dose-dependent manner. However, the effect of IL-17 was always less than that of IL-1.

These results were first obtained with optimal concentrations, which may not represent the situation found in vivo. Accordingly, stimulation was performed using low concentrations of IL-17 (1 ng/ml) and IL-1β (1 pg/ml) in combination. In such cultures, a

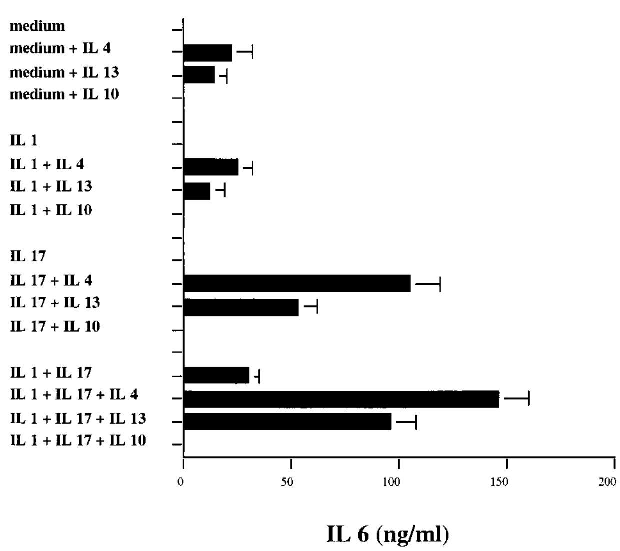


FIGURE 3. Effect of IL-4, IL-10, and IL-13 on the synergistic effect of IL-1β and IL-17 on IL-6 production. RA synoviocytes were cultured with suboptimal concentrations of IL-1β (1 pg/ml) with or without IL-17 (1 ng/ml) in the presence of 50 ng/ml of IL-4, IL-10, or IL-13. After 7 days of culture, IL-6 levels were measured by ELISA.

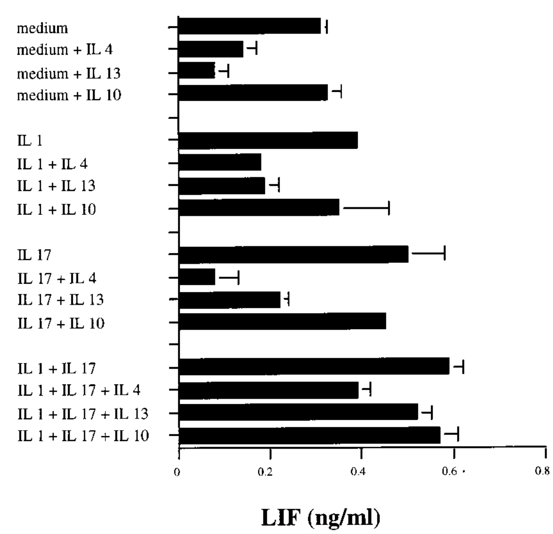


FIGURE 4. Effect of IL-4, IL-10, and IL-13 on the effect of IL-1β and IL-17 on LIF induction. RA synoviocytes were cultured with suboptimal concentrations of IL-1β (1 pg/ml) with or without IL-17 (5 ng/ml) in the presence of 50 ng/ml of IL-4, IL-10, or IL-13. After 7 days of culture, LIF levels were measured by ELISA.

Table III. *Induction of IL-6 and LIF production by endogenous IL-17 produced by RA synovium pieces*[a]

Activators	IL-6 (ng/ml)	LIF (ng/ml)
Medium	0	0.05 ± 0.01
IL-17	15.49 ± 2.41	0.98 ± 0.07
IL-17 + mAb5	0	0.06 ± 0.01
IL-17 + MX1	14.63 ± 3.8	ND
IL-1	154.60 ± 13.48	1.64 ± 0.20
IL-1 + mAb5	145.95 ± 22.5	1.62 ± 0.18
RA 54 supernatant	151.3 ± 11.09	1.20 ± 0.04
RA 54 supernatant + mAb5	52.7 ± 9.10	0.54 ± 0.01

[a] Cytokines (IL-1β, 100 pg/ml, or IL-17, 50 ng/ml) or synovium piece supernatants were preincubated in the presence or absence of 1 μg/ml of a control mAb (MX1) or 1 μg/ml of anti-IL-17 (mAb5). The mixture was incubated with synoviocytes for 12 h. Plates were washed before addition of fresh medium. IL-6 and LIF levels were determined by ELISA in the 48-h supernatants.

synergistic effect of IL-1 and IL-17 was observed on the production of IL-6, whereas an additive effect was observed for LIF production. Similarly, whereas neither IL-17 nor TNF-α alone had any effect on the secretion of GM-CSF, the combination of the two cytokines induced its production by synoviocytes (19). In keeping with the in vivo relevance, these results indicate that cytokines produced by monocytes (IL-1 or TNF-α) and T cells (IL-17) can act together on synoviocytes at the low levels that can most probably be achieved in vivo. Such additive or even synergistic effects explain the important reduction of the IL-6- and LIF-inducing activity present in RA synovium supernatants with a blocking anti-IL-17 Ab (Table III).

The mode of action of IL-17 on IL-1 effect remains to be clarified. A differential effect on the induction of cytokine receptors may lead to synergy. Indeed, the combination of IFN-γ and TNF-α was found to induce TNF-Rα expression, leading to increased response to TNF-α (26). Similar findings were obtained for the effect of IL-10 on IL-2R expression by B cells (27). Such additive or synergistic effects could be expected because the respective IL-1 and IL-17 receptors appear to belong to different families (28). A complete characterization of the components of the multichain IL-17R is in progress. Further studies will thus be required to clarify the molecular basis of the interaction between IL-1 and IL-17.

IL-17 acts as a Th1 cytokine

The proinflammatory effects of IL-17 suggest its classification as a Th1 cytokine. This includes its effects on synoviocytes and the maturation of neutrophils. Most of the T cell clones derived from RA synovium have been classified as Th1 (29). Our recent studies suggest that some IFN-γ-producing RA synovium T cell clones also produce biologically active IL-17 (unpublished data). Such findings, combined with the demonstration of IL-17 production by RA synovium, are in line with the classification of IL-17 as a Th1 cytokine. This subset of T cells can then act directly on mesenchymal cells, leading to an increased proinflammatory pattern and mediating matrix destruction (20). It could be expected that such a concept also applies to other Th1-mediated chronic diseases such as multiple sclerosis, psoriasis, and diabetes (17). The exact proportion of IL-17-producing T cells and their function remain to be determined. Conversely, control of such an effect may lead to a therapeutic anti-inflammatory property.

Effects of Th2 cytokines

The Th2 cytokines IL-4, IL-13, and IL-10 have been classified as anti-inflammatory on the basis of their inhibitory effect on the production of IL-1, TNF-α, IL-6, and IL-8 by monocytes and also by synovium targets (11, 12, 14–17). We studied the effects of these cytokines on the production of IL-6 and LIF by RA synoviocytes stimulated by IL-1 and IL-17. Both IL-4 and IL-13 enhanced by a modest 30% the IL-1- and IL-17-induced production of IL-6. In contrast, they inhibited the production of LIF by 60 to 70%. IL-13 was in general less potent than IL-4. The enhancing effect of IL-4 or IL-13 was more potent with low concentrations of IL-17 than with IL-1β.

Effects of IL-10

Surprisingly, the effects of IL-10 on IL-6 and LIF production were limited, in line with previous reports (7). IL-10 had no effect on LIF production, and a modest inhibitory effect of IL-6 production was found in response to IL-17 and/or IL-1. This is in contrast with its potent inhibitory effect on the secretion of proinflammatory cytokines by monocytes (15). Endogenous IL-10 has been shown to be produced by RA synovium acting as an anti-inflammatory cytokine (12). In addition, T cell clones from RA synovium also produced IL-10 (29). Because of its high endogenous production, addition of IL-10 had limited effect on cytokine production by RA synovium (30). On isolated synoviocytes, contrasting destructive and protective effects have been described. On one hand, IL-10 was found to down-regulate collagen I expression by fibroblasts while enhancing the production of collagenase and stromelysin (31). On the other hand, enhancement of tissue inhibitor of metalloproteinases (TIMP) production would limit destruction (32). In vivo studies will clarify the net effect of IL-10 on these targets.

Differential effect on LIF and IL-6 production

The opposite effects of IL-4 and IL-13 on IL-6 and LIF production further demonstrate the complexity of the cytokine network. Both IL-6 and LIF use the common gp130 receptor chain to activate target cells (33). However, they are differently regulated by IL-4 and IL-13 when combined with IL-17 and IL-1, findings that extend our previous results with IL-1 (7). This effect on synoviocytes contrast with the common inhibitory effect of IL-4 and IL-13 observed with monocytes and whole synovium (7, 14, 16, 17). In addition, it should be underlined that the classification of IL-6 as a proinflammatory cytokine remains a matter of debate. Through the induction of acute phase proteins, IL-6 also acts as a anti-inflammatory cytokine (34). Such an effect appears to be further enhanced by IL-4 and IL-13 when cells are first exposed to in vivo proinflammatory signals such as IL-17 and IL-1 combined at low concentrations. This would further enhance their anti-inflammatory properties at the site of inflammation.

In conclusion, IL-17 appears to be a T cell cytokine produced by the RA synovium, which acts directly on synoviocytes, leading to an enhanced proinflammatory secretion profile when combined with monocyte-derived signals. Accordingly, control of the production and action of IL-17 may represent a therapeutic target for reducing the enhancing effect of monocyte-derived cytokines. Among the available molecules of interest, some Th2 cytokines may control part of the consequences of such interaction.

References

1. Harris, E. D. 1990. Rheumatoid arthritis: pathophysiology and implications for therapy. *N. Engl. J. Med. 322:1277.*
2. Arend, W. P., and J.-M. Dayer. 1990. Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. *Arthritis Rheum. 33:305.*
3. Dayer, J.-M., B. Beutler, and A. Carami. 1985. Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E_2 production by human synovial cells and dermal fibroblasts. *J. Exp. Med. 162:2163.*
4. Dayer, J.-M., B. de Rochemonteix, B. Burrus, S. Demczuk, and C. A. Dinarello. 1986. Human recombinant interleukin 1 stimulates collagenase and prostaglandin E_2 production by human synovial cells. *J. Clin. Invest. 77:645.*
5. Guerne, P.-A., B. L. Zuraw, J. H. Vaughan, D. A. Carson, and M. Lotz. 1989. Synovium as a source of interleukin 6 in vitro: contribution to local and systemic manifestations of arthritis. *J. Clin. Invest. 83:585.*

6. Hamilton, J. A., E. L. Filonzi, and G. Ianches. 1993. Regulation of macrophage colony-stimulating factor (M-CSF) production in cultured human synovial fibroblasts. *Growth Factors 9:157.*
7. Dechanet, J., J. L. Taupin, P. Chomarat, M. C. Rissoan, J. F. Moreau, J. Banchereau, and P. Miossec. 1994. Interleukin-4 but not interleukin-10 inhibits the production of leukemia inhibitory factor by rheumatoid synovium and synoviocytes. *Eur. J. Immunol. 24:3222.*
8. Okamoto, H., M. Yamamura, Y. Morita, S. Harada, H. Makino, and Z. Ota. 1997. The synovial expression and serum levels of interleukin-6, interleukin-11, leukemia inhibitory factor, and oncostatin M in rheumatoid arthritis. *Arthritis Rheum. 40:1096.*
9. Firestein, G. S., J. Alvaro-Gracia, and R. Maki. 1990. Quantitative analysis of cytokine gene expression in rheumatoid arthritis. *J. Immunol. 144:3347.*
10. Miossec, P., M. Navilliat, A. Dupuy d'Angeac, J. Sany, and J. Banchereau. 1990. Low levels of interleukin 4 and high levels of transforming growth factor β in rheumatoid synovitis. *Arthritis Rheum. 33:1180.*
11. Miossec, P., J. Briolay, J. Dechanet, J. Wijdenes, H. Martinez-Valdez, and J. Banchereau. 1992. Interleukin 4 inhibits ex vivo production of proinflammatory cytokines and immunoglobulins by rheumatoid synovitis. *Arthritis Rheum. 35:874.*
12. Katsikis, P. D., C. Q. Chu, F. M. Brennan, R. N. Maini, and M. Feldmann. 1994. Immunoregulatory role of interleukin 10 in rheumatoid arthritis. *J. Exp. Med. 179:1517.*
13. Mosmann, T. R., and R. L. Coffman. 1989. T_{H1} and T_{H2} cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol. 7:145.*
14. Cheung, D. L., P. H. Hart, G. H. Vitti, G. A. Whitty, and J. A. Hamilton. 1990. Contrasting effects of interferon-gamma and interleukin-4 on the interleukin-6 activity of stimulated human monocytes. *Immunology 71:70.*
15. de Waal Malefyt, R., J. Abrams, B. Bennett, C. G. Figdor, and J. E. de Vries. 1991. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med. 174:1209.*
16. de Waal Malefyt, R., C. G. Figdor, R. Huijbens, S. Mohan-Peterson, B. Bennet, J. Culpepper, W. Dang, G. Zurawski, and J. E. de Vries. 1993. Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. *J. Immunol. 151:6370.*
17. Miossec, P., and W. B. Van den Berg. 1997. The Th1/Th2 cytokine balance in arthritis. *Arthritis Rheum. 40:2105.*
18. Yao, Z., S. L. Painter, W. C. Fanslow, D. Ulrich, B. M. Macduff, M. K. Spriggs, and R. J. Armitage. 1995. Human IL-17: A novel cytokine derived from T cells. *J. Immunol. 155:5483.*
19. Fossiez, F., O. Djossou, P. Chomarat, L. Flores-Romo, S. Ait-Yahia, C. Maat, J. J. Pin, P. Garrone, E. Garcia, S. Saeland, D. Blanchard, C. Gaillard, B. Das Mahapatra, E. Rouvier, P. Golstein, J. Banchereau, and S. Lebecque. 1996. T cell Interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J. Exp. Med. 183:2593.*
20. Attur, M. G., R. N. Patel, S. B. Abramson, and A. R. Amin. 1997. Interleukin-17 up-regulation of nitric oxide production in human osteoarthritis cartilage. *Arthritis Rheum. 40:1050.*
21. Arnett, F. C., S. M. Edworthy, D. A. Bloch, D. J. McShane, J. F. Pries, N. S. Cooper, L. A. Healey, S. R. Kaplan, M. H. Liang, H. S. Luthra, T. A. Medsger, D. M. Mitchell, D. H. Nenstadt, R. S. Pinals, J. G. Schaller, J. T. Sharp, R. L. Wilder, and G. C. Hunder. 1988. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum. 31:315.*
22. Abrams, J. S., M.-G. Roncarolo, H. Yssel, U. Andersson, G. J. Gleich, and J. E. Silver. 1992. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol. Rev. 127:5.*
23. Taupin, J. L., N. Gualde, and J. F. Moreau. 1997. A monoclonal antibody based ELISA for quantitation of human leukaemia inhibitory factor. *Cytokine 9:112.*
24. Firestein, G. S., and N. J. Zvaifler. 1990. How important are T cells in chronic rheumatoid synovitis? *Arthritis Rheum. 33:768.*
25. Chomarat, P., M. C. Rissoan, J. J. Pin, J. Banchereau, and P. Miossec. 1995. Contribution of IL-1, CD14, and CD13 in the increased IL-6 production during monocyte-synoviocyte interactions. *J. Immunol. 155:3645.*
26. Ruggiero, V., J. Tavernier, W. Fiers, and C. Baglioni. 1986. Induction of the synthesis of tumor necrosis factor receptors by interferon-γ. *J. Immunol. 136:2445.*
27. Fluckiger, A.-C., P. Garrone, I. Durand, J. P. Galizzi, and J. Banchereau. 1993. IL-10 upregulates functional high affinity IL-2 receptors on normal and leukemic B lymphocytes. *J. Exp. Med. 178:1473.*
28. Yao, Z., W. C. Fanslow, M. F. Seldin, A. M. Rousseau, S. L. Painter, M. R. Comeau, J. I. Cohen, and M. K. Spriggs. 1995. *Herpesvirus saimiri* encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity 3:811.*
29. Quayle, A. J., P. Chomarat, P. Miossec, J. Kjeldsen-Kragh, O. Førre, and J. B. Natvig. 1993. Rheumatoid inflammatory T-cell clones express mostly Th1 but also Th2 and mixed (Th0-like) cytokine patterns. *Scand. J. Immunol. 38:75.*
30. Chomarat, P., J. Banchereau, and P. Miossec. 1995. Differential effects of interleukins 10 and 4 on the production of interleukin-6 by blood and synovium monocytes in rheumatoid arthritis. *Arthritis Rheum. 38:1046.*
31. Reitamo, S., A. Remitz, and J. Uitto. 1994. Interleukin-10 modulates type I collagen and matrix metalloprotease gene expression in cultured human skin fibroblasts. *J. Clin. Invest. 94:2489.*
32. Lacraz, S., L. P. Nicod, R. Chicheportiche, H. G. Welgus, and J. M. Dayer. 1995. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. *J. Clin. Invest. 96:2304.*
33. Kuropatwinski, K. K., C. De Imus, D. Gearing, H. Baumann, and B. Mosley. 1997. Influence of subunit combinations on signaling by receptors for oncostatin M, leukemia inhibitory factor, and interleukin-6. *J. Biol. Chem. 272:15135.*
34. Tilg, H., C. A. Dinarello, and J. W. Mier. 1997. IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. *Immunol. Today 18:428.*