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IL-17 Is Produced by Some Proinflammatory Th1/Th0 Cells But Not by Th2 Cells[1]

Tanja Aarvak,[2]* Martine Chabaud,[†] Pierre Miossec,[†] and Jacob B. Natvig*

IL-17 is defined as a proinflammatory cytokine and produced by activated CD4^{+} T cells. In rheumatoid arthritis synovial tissue, high levels of IL-17 contribute to IL-6 production by synoviocytes. The present study was performed to see whether Th cells that produce IL-17 are associated with the Th1, Th2, or Th0 subset. Thirty-three CD4^{+}, $\alpha\beta^+$ T cell clones were developed from synovial membranes and synovial fluid of rheumatoid arthritis patients. Thirteen clones were defined as Th1 since they produced IFN-γ but not IL-4, and four clones were defined as Th0 type that produced both IL-4 and IFN-γ. Sixteen clones were defined as Th2 since they produced high levels of IL-4 and/or IL-10 but not IFN-γ. IL-17 was measured in a bioassay, where IL-6 production from synoviocytes was a measurement for IL-17 activity in the presence and absence of blocking anti-IL-17 mAb. Three Th1 clones and two Th0 clones produced IL-17. In contrast, none of the sixteen Th2 clones analyzed produced IL-17. In addition, six Th2 clones were further cultured in conditions that induced a switch to Th1 type. Induction of this Th1 phenotype also led to production of IL-17 in two of these clones. The results demonstrate that some cells of the Th1/Th0 phenotype produce IL-17 but not cells of the Th2 phenotype. Thus, IL-17 may define a new subset of T cells, and IL-17 production appears to be a mechanism for Th1/Th0 cells, the most frequent Th subtype present in the rheumatoid synovium, to contribute to the local inflammatory reactions. *The Journal of Immunology,* 1999, 162: 1246–1251.

I nterleukin-17 has recently been characterized as the human counterpart of mouse CTLA-8 and has been shown to stimulate epithelial, endothelial, and fibroblastic cells to induce expression of IL-6, IL-8, granulocyte-macrophage-CSF and PGE$_2$ (1, 2). CD4^{+} Th cells produce IL-17, but it is still uncertain whether all CD4^{+} cells or a subset of these produce IL-17. Th cells can be divided into Th1 and Th2 subtypes according to their cytokine profiles and have different functional properties (3). Th1 cells produce IFN-γ, IL-2, and TNF-β, which induce cellular immunity and production of proinflammatory cytokines. Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13 and favor B cell-mediated humoral immunity and antiinflammatory cytokine patterns (4). Various membrane markers have been proposed as being able to specifically dissociate Th1 from Th2 types. In particular, the chemokine receptor CCR3 appears to be selectively expressed on Th2 but not Th1 cells (5, 6). Th1- and Th2-type responses not only play different roles in protection but also can promote different immunopathological reactions. Chronic inflammatory diseases are often Th1 mediated (7), and, similarly, IgE-associated allergic diseases are often related to Th2 responses.

Rheumatoid arthritis (RA)[3] is characterized by dense lymphoid infiltrates in the synovial membrane associated with progressive tissue destruction (8, 9). The synovium is highly infiltrated by CD4^{+} Th cells (10) that can influence the disease mechanisms in RA by the regulatory role of Th1 and Th2 cells (11–13). In the RA synovium, Th1 cells are predominant, but Th2 cells and their common precursor Th0 cells can also be found (14–16). Despite a high number of T cells in the synovium, concentrations of the T cell cytokines IFN-γ and IL-4 are quite low, and how these T cells contribute to the inflammation and joint damage is not completely understood (17, 18).

Recent studies have found a high concentration of IL-17 expressed and produced by the RA synovium (19, 20). Moreover, proinflammatory cytokines like IL-1, IL-6, and TNF-α can be detected in high levels in the inflammatory synovium (21, 22), and both TNF-α and IL-1, when in combination with IL-17, increase IL-6 secretion by synoviocytes in a synergistic fashion (19). To better understand the local inflammatory processes in RA synovium, we have investigated the cellular origin of the new proinflammatory cytokine IL-17, in relation to IFN-γ, IL-4, and IL-10. The aim of this study was to clarify whether Th cells in the synovium produce IL-17 and whether this production can be associated to a particular Th cell subset. Therefore T cell clones were developed from synovial membranes and fluid of RA patients and analyzed for their capacity to secrete IL-17, IFN-γ, IL-4, and IL-10 after stimulation. In addition, we analyzed for conditions able to induce IL-17 secretion.

The results demonstrate on a clonal level that IL-17 is produced by some CD4^{+} T cells that are exclusively of the Th1/Th0 type and not of the Th2 type (23). Consequently, these IL-17-producing Th1/Th0 cells may define a new subset of proinflammatory Th1/Th0 cells. In line with these observations, IL-17 production can also be induced in some cells that have switched from Th2 to Th1.

Table I. *T cell cloning protocols*

Donors: RA Patients	Origin	Primary Stimulus	No. of Cells per Well	Cloned with:	Cloning Frequency (% positive wells)	No. of Clones
KB	ST	BCG	0.5	PHA	0.8	3
KB	ST	PHA	0.5	PHA	13	13
SHK	SF	BCG	0.3	PHA	2.5	2
NHE	ST	BCG	1	PHA	2.8	4
NHE	ST	PHA	0.3	PHA	2.2	3
TFH	ST	PHA	0.5	PHA	2.4	8

Materials and Methods

Patients

Samples of synovial tissue (ST), synovial fluid (SF), and peripheral blood were obtained from two female RA and two female JRA patients hospitalized at the Oslo Sanitetsforenings Rheumatism Hospital. TFH is a patient (born in 1947) diagnosed as typical seropositive RA in 1979 (HLA-DRB1*0404). ST was obtained from the left elbow, and TFH had been treated with prednisolon, ciclosporin, and NSAID the year before the surgery. KB (born in 1933) was diagnosed as seronegative RA in 1979 (HLA-DRB1*0404). The last year before surgery, KB was treated with NSAID. The ST sample was obtained from a right elbow synovectomy. SHK is a patient (born in 1969) with seronegative polyarticular JRA from 1983 (HLA-DRB1*0401) and was treated with hydroxychlorochin and sulfasalazin. SF from the right knee was obtained 6 mo after pregnancy, and SHK did not receive any medication other than NSAID during the pregnancy. After the pregnancy she again started with hydroxychlorochin and sulfasalazin and subsequently with metotrexate. The patient NHE (born in 1965) was diagnosed as seronegative pauciarticular JRA in 1974 (HLA-DRB1*0401). NHE was treated with hydroxychlorochin, NSAID, and later on penicillamine. She was also treated for iridocyclitis. ST samples were obtained from synovectomy of the left elbow. All four patients fulfilled the criteria of the American College of Rheumatology (ACR) for RA and JRA, respectively (24).

Preparation of mononuclear cells

SF pretreated for 20 min with 10 U/ml hyaluronidase at 37°C, or pool of buffy coat (from 10 normal blood donors at the blood bank, Ullevål Hospital) were layered on Isopaque-Ficoll separation media (Lymphoprep, Nycomed, Oslo, Norway) and centrifuged at $320 \times g$ for 25 min. The cells were washed three times with HBSS and resuspended at a concentration of 1×10^6/ml in RPMI 1640 supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% pooled human serum. This medium was used for culturing T cells throughout the study. ST were cut into small pieces and digested with 4 mg/ml collagenase (Sigma, St. Louis, MO) in PBS (Life Technologies, Grand Island, NY) for 1.5 h at 37°C. After centrifugation at $320 \times g$ for 10 min, the cells were washed three times with HBSS and cultured in RPMI 1640 overnight to remove adherent cells. Tissue debris was removed, and the supernatant was laid on Isopaque-Ficoll separation media and separated and washed as described above.

T cell cloning

The protocol for stimulation of lymphocytes from ST and SF, the subsequent T cell cloning, and the number of viable clones from each patient are given in Table I. Mononuclear cells (1.5×10^6) from ST and SF were initially stimulated with 2.5 mg/ml PHA (Sigma) or 10 μg/ml BCG (culture fluid was a kind gift from H. Wiker, Institute of Immunology and Rheumatology, Oslo, Norway). After 3 days, and subsequently every fourth day, the cell lines were fed with 20 U/ml recombinant IL-2 (Boehringer Mannheim, Mannheim, Germany). Cloning was performed on day 14 by limiting dilution (0.3–1 cells/well) in 20-μl wells (Terasaki; Nunc, Roskilde, Denmark), using 2×10^4 irradiated allogenic PBMC, 2.5 mg/ml PHA, and 20 U/ml IL-2. Growing T cell clones were expanded after 8 days to 96-well plates (Nunc) in fresh complete medium containing 20 U/ml IL-2, 2.5 μg/ml PHA, and 1×10^6 irradiated allogenic PBMC and later to 24-well plates (Falcon, Becton Dickinson, Rutherford, NJ). T cell clones were feed twice weekly and restimulated every 12 days with PHA, irradiated allogenic PBMC, and IL-2.

T cell clones were tested for proliferative capacity in a standard proliferation assay. Cloned T cells (2×10^4 in RPMI 1640) were stimulated with 10 μg/ml BCG or 2.5 μg/ml PHA in the presence of 7×10^4 autologous PBMC. After 72 h of incubation, 0.5 mCi/well tritiated thymidine was added, and the cells were harvested 18 h later.

Stimulation of T cell clones

T cell clones were stimulated with anti-CD3 (HIT3a, PharMingen, San Diego, CA). Anti-CD3 diluted in PBS (0.1 μg/ml) was coated on 24-well plates (Becton Dickinson) at 37°C for 3 h, and, after incubation, the wells were washed twice in PBS. Cloned cells (1×10^6 cells/ml in medium) were added to each well at 1.5×10^6 cells/well. As a control, cloned cells were added to wells that had been coated with PBS only. The clones had been stimulated with PHA 12 days before the assay. After 24 h incubation, the cells and supernatants were collected and centrifuged at $320 \times g$ for 10 min and stored at -20°C until cytokine quantification could be performed. In parallel, T cell clones were set up in 96-well plates (Nunc) in triplicate, with 50 μl/well at the same concentration. After 24 h incubation, the cells were pulsed with 0.5 mCi of tritiated thymidine (Amersham, Amersham, U.K.) for 18 h after incubation with anti-CD3. Incorporation of tritiated thymidine was measured by a beta counter. The clonality of the T cell clones was assured by cloning down to 0.3 cells per well and the low cloning frequency (Table I).

Phenotyping of T cell clones

Cells were phenotyped using a standard direct immunofluorescent technique (25) and analyzed with a FACscan (Becton Dickinson). mAbs used for staining were as follows: anti-TCR$\alpha\beta$ (FITC-T10B9.1A-31), anti-TCRγ/δ (PE-B1.1), anti-CD4 (PE-RPA-T4), anti-CD8 (FITC-HIT8a), anti-CD28 (PE-CD28.2), anti-CD27 (PE-M-T271), anti-CD57 (FITC-NK-1) (all PharMingen), anti CD45RB (FITC-PD7/26) (DAKO, Glostrup, Denmark). Anti-CCR3 (7B11) was kindly provided from Dr. Walter Newman and Dr. Paul Ponath (LeukoSite, Cambridge, MA).

HLA-DR typing

DNA was prepared from T cell clones using GenomicPrep Blood DNA Isolation Kit (Pharmacia Biotech, Piscataway, NJ). The samples were kindly analyzed by Hanne Akselsen at the Institute of Transplantation Immunology, the National Hospital, Norway, with Amplicore HLA DRB Typing Kit (Dynal, Oslo, Norway) and further subtyped with Dynal DRB1–04 SSP.

Measurements of cytokine levels

The capacity of T cell clones to produce IL-17 was measured in a bioassay using IL-6 production from synoviocytes. To isolate synoviocytes, synovium pieces obtained from patients with RA were digested as described above under preparation of mononuclear cells. 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 appeared to be 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.

Synoviocytes (10^4 cells/well) were incubated in 96-well plates (Nunc) in a final volume of 200 μl of a-MEM (Life Technologies) with 2 mM L-glutamine, 100 U/ml penicillin, 50 mg/ml gentamicin, 20 mM HEPES buffer, and 2% Ultroser (Life Technologies). Samples were preincubated at 37°C for 30 min with 1 μg/ml of anti-IL-17 mAb5 (Schering-Plough Research Institute, Kenilworth, NJ). Following the preincubation step, IL-17 (50 ng/ml) or 1:2 dilution T cell 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.

IL-6 levels were measured by two-site sandwich ELISA as previously described (26). Briefly, supernatants or serial dilutions of IL-6 standards

(Schering-Plough) were incubated for 60 min at 37°C in 96-microtiter plates (Nunc) coated overnight at 4°C with the mouse 39C3 anti-IL-6 mAb (1 μg/ml), and saturated 90 min at 20°C with PBS 5% bovine albumin. 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 per-oxidase-coupled streptavidin and development with orthophenylenedia-mine (Sigma), the plates were read at 492 nm.

IFN-γ (Schering-Plough), IL-4, and IL-10 (R&D Systems, Abington, U.K.) levels were also measured by a two-site sandwich ELISA using pairs of anti-IFN, anti-IL-4, and anti-IL-10 mAbs. Mouse A35 anti-human IFN, MAB604 anti-IL-4, and MAB217 anti-IL-10 mAb were used for coating, and biotinylated mouse B27 anti-human IFN, BAF204 anti-IL-4, and BAF217 anti-IL-10 mAb were used for detection. After subsequent incu-bation with a streptavidin peroxidase complex and development with or-thophenylenediamine, the OD were measured at 492 nm.

Induction of IL-17 production during Th1 development

To analyze whether IL-17 could be induced during Th1 development, Th2 clones negative for the production of IL-17 were cultured in Th1 conditions and then analyzed for IL-17 and IFN-γ production. Th1 clones were gen-erated as previously described (27), by stimulating with irradiated PBMC and 2.5 μg/ml PHA in the presence of 2 ng/ml IL-12 (PharMingen) and 200 ng/ml neutralizing anti-IL-4 Ab (PharMingen). The clones were fur-ther cultured and stimulated as described above.

Statistical calculations

Results are expressed as mean ± SEM. Differences between groups were compared with Mann-Whitney U test and Fisher exact test. A p value less than 0.05 was considered statistically significant.

Results

Characterization of RA T cell clones

To assay, on a clonal level, IL-17 production by T cells, 33 T cell clones developed from ST and SF (Tables I and II) were first analyzed for Th1- and Th2-type cytokines after stimulation with anti-CD3. Unstimulated clones did not proliferate and did not spontaneously produce any of the cytokines tested for.

Seventeen clones from three patients (NHE, SHK, and TFH) secreted IFN-γ (2–15 ng/ml) (Table II). Thirteen of these clones produced IFN-γ without producing any IL-4, which is typical for the Th1 type. Five of these Th1 clones secreted small levels of IL-10. Four clones (NHEP2, NHE3, TFH5, and KBc18) produced both IFN-γ and IL-4 and were therefore classified as Th0 type. Three of these Th0-type clones also secreted IL-10.

Sixteen Th2 clones from patient KB produced IL-4 and/or IL-10 (0.1–3.0 ng/ml) and not IFN-γ and were accordingly classified as Th2 clones. Of these Th2 clones, eight produced IL-10. The levels of IFN-γ (6.3 ± 1.1 ng/ml) produced by the Th1 cells were sig-nificantly higher than IL-4 (1.1 ± 0.2 ng/ml) $p = 0.003$ and of IL-10 (0.3 ± 0.1) $p < 0.0001$ produced both by the Th1 and Th2 (Mann-Whitney U test).

IL-17 production by different subtypes of CD4+ T cells

To answer the question whether Th1, Th0, or Th2 clones produce IL-17, 4 Th0, 13 Th1, and 16 Th2 clones were analyzed for IL-17 activity. IL-17 activity was defined as the concentration of IL-6 produced by synoviocytes upon stimulation by culture supernatant. IL-17 activity was found in five clones that all secreted IFN-γ (Table II and Fig. 1). Three of these IL-17 producers were classi-fied as Th1 (SHK4, NHE7, and TFH7) since they did not produce IL-4, and two as Th0 (NHE3 and TFH5) since they also secreted IL-4 (1.1 and 0.2 ng/ml, respectively). In contrast, none of 16 Th2 clones produced any IL-17. IL-17 is therefore produced only by Th1- and Th0-type clones but not by the Th2. This association of IL-17 production with Th1/Th0 clones and not Th2 clones is sta-tistically significant in Fisher exact test, $p = 0.04$ (Table III).

In total, 5 of 17 (30%) of the Th0/Th1 clones produced IL-17 (Table III). The activity level of IL-17 varied between the clones.

Table II. *Cytokine profile of 33 CD4+ αβ-TCR clones*

Clones	IFNγ (ng/ml)	IL-4 (ng/ml)	IL-10 (ng/ml)	IL-17 Activity[a]	Classification
NHEP2	15.3	2.5	0.7	0	Th0
NHE7	13.3	0	0.1	64	Th1
NHEP1	11.7	0	0.9	0	Th1
TFH7	9.9	0	0	8	Th1
TFH4	9.5	0	0.1	0	Th1
TFH6	9.2	0	0	0	Th1
NHEP6	8.9	0	0	0	Th1
TFH10	7.4	0	0	0	Th1
NHE6	6.8	0	0.1	0	Th1
NHE3	5.0	1.1	0.1	10	Th0
SHK4	3.9	0	0.1	4	Th1
NHE5	2.2	0	0	0	Th1
SHK6	1.8	0	0	0	Th1
TFH5	1.2	0.2	0	19	Th0
TFH2	0.4	0	0	0	Th1
KB18c	0.3	0.7	0.2	0	Th0
TFH9	0.1	0	0	0	Th1
KB26c	0	3.0	0	0	Th2
KB38c	0	2.2	0.1	0	Th2
KB17b	0	2.0	0.3	0	Th2
KB33c	0	1.7	0	0	Th2
KB30c	0	1.2	1.0	0	Th2
KB8c	0	1.1	0	0	Th2
KB34c	0	1.0	0.4	0	Th2
KB1	0	0.8	0.1	0	Th2
Kb10b	0	0.8	0.1	0	Th2
Kb12c	0	0.8	0	0	Th2
Kb6b	0	0.4	0	0	Th2
KB15c	0	0.4	0	0	Th2
KB20b	0	0.4	0	0	Th2
KB4	0	0.1	0	0	Th2
KB13c	0	0	0.3	0	Th2
KB7c	0	0	0.1	0	Th2

[a] One microgram per milliliter of mAb5 was able to completely inhibit the IL-6 production in response to 50 ng/ml of rIL-17. The IL-17 activity 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. Detection limit of IL-6 was 50 pg/ml.

One Th1 clone (NHE7) produced a very high activity level of IL-17, and this clone also produced the highest level of IFN-γ (13.3 ng/ml) while the concentration of IL-4 was low. The four other Th0 and Th1 clones produced intermediate levels of IL-17, and IFN-γ production in these clones was lower than in the NHE7 clone (between 9.9 and 1.2 ng/ml).

In addition to IL-4 that was found in two IL-17 producing clones, IL-10 was also frequently synthesized in these clones. Three clones (60%) produced IL-10 (Table III), which is about the
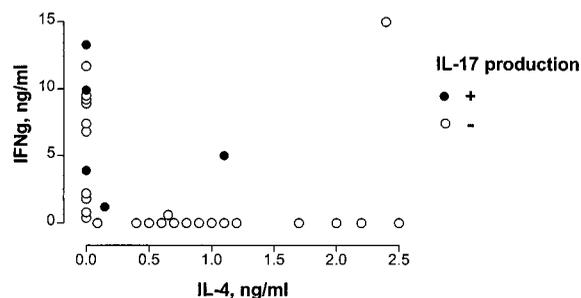

FIGURE 1. Distribution of different CD4+ subsets according to their cytokine production. T cell clones were stimulated with immobilized anti-CD3 for 24 h. Cytokine measurements for IL-17 activity were performed in a bioassay in presence and absence of blocking anti-IL-17 mAb. IL-4 and IFN-γ were analyzed with ELISA.

Table III. *Number of clones that secrete various cytokines compared to different CD4⁺ subsets*

CD4⁺ Subsets	IL-17	IFN-γ	IL-4	IL-10	Total
Th1/Th0[a]	+	5	2	3	5
Th1/Th0[a]	−	12	2	6	12
Th2[a]	−	0	14	9	16

[a] The association of IL-17 production with Th1/Th0 cells and not Th2 cells is statistically significant using Fischer exact test ($p = 0.04$).

same proportion as found in the Th1/Th0 clones that did not produce IL-17. This shows that IL-17-positive clones produced IL-10 with about the same frequency as Th1/Th0 cells negative for IL-17 production, and Th2 cells. Moreover, the frequent production of IL-4 and IL-10 indicates that IL-17 production is an event that occurs often in the differentiation from Th0 to Th1, as well as in the Th1 state (Fig. 1).

IL-17 production in relation to expression of different T cell membrane markers

To further characterize the IL-17-producing Th1/Th0 cells, the clones were screened for expression of the membrane markers CCR3, CD28, CD45RB, CD57, and CD27 (Table IV). Of the IL-17-producing Th1/Th0 clones, the three Th1 clones were CCR3 negative. The two Th0 clones, producing low levels of IL-4 in association with significant levels of IFN-γ, were CCR3 positive as were most of the IL-4-producing Th2 clones. Table IV shows that there was no significant association between the IL-17-producing clones and the other markers screened for. It should be noticed, however, that all IL-17-secreting clones were CD27 negative.

Induction of Th1 clones and IL-17 production

Experiments were performed to see whether Th2 clones that did not produce any IL-17 could be switched to the Th1 phenotype with subsequent IL-17 production. Three Th2 clones (KBc8, KBc13, and KBb10) were cultured in a Th1 environment with IL-12 and anti-IL-4. After 4 wk with IL-12 and anti-IL-4, these clones changed their phenotype from Th2 to Th1 and started to secrete IFN-γ and lost the IL-4 secretion. One of the three clones (KBb10) also produced IL-17 after this shift in phenotype (Fig. 2). In addition, one of three other clones that switched from Th2 to Th1 after continuous stimulation by IL-2 also started to produce IL-17 (28). Thus, altogether two of six clones started to produce IL-17 after switching from Th2 to Th1. These results show that, during Th1 development, a proportion of the cells producing IFN-γ also produce IL-17.

Discussion

IL-17 was first described as a cytokine produced specifically by activated T cells (29). IL-17 is a proinflammatory cytokine because

it induces the production of IL-6, granulocyte-macrophage-CSF, PGE₂, and leukemia inhibitory factor (LIF) (1). However, the classification of IL-17 production in relation to the Th1 and Th2 subsets is still pending. Since IL-17 has a direct effect on synoviocytes, IL-17 appears to represent a way for T cells to induce inflammation, in particular, in association with low levels of monocyte-derived cytokines (19). The results presented in this report demonstrate on a clonal level that only the Th1 and Th0 subsets produce IL-17, but not the Th2 subset. Five of 17 Th1 or Th0 clones produced IL-17, while none of the 16 Th2 clones produced IL-17.

To further characterize the IL-17-producing Th1/Th0 cells, the clones were analyzed for expression of different membrane markers and correlated to the IL-17 production. This included the recently described CCR3 receptor that is selectively expressed by the Th2 population and not by the Th1 (5), as well as other markers. Of the IL-17-producing Th1/Th0 cell, the three Th1 clones were CCR3 negative. The two Th0 clones, producing low levels of IL-4 in association with significant levels of IFN-γ, were CCR3 positive in addition to most of the Th2 clones. These studies show that CCR3 behaves more as a marker for IL-4 secretion rather than a perfect Th2 marker. There was otherwise no significant difference between the IL-17-producing Th1/Th0 clones and the other clones in their expression of CCR3 and the other membrane markers tested for. Thus IL-17 appears to be a key marker to identify these cells. Furthermore, all the IL-17-producing clones were negative for expression of CD27. A previous report suggests that the CD27⁻ CD4⁺ T cell is linked to production of IL-4 (30), which is in contrast to our findings where only weak IL-4 production is found in two of the five IL-17-producing clones. Thus, loss of CD27 expression in these clones is, rather, related to their memory phenotype and not the production of IL-4.

In addition, to analyze whether IL-17 could be induced during Th1 development, Th2 clones were cultured in a Th1 environment. IL-12 production by macrophages has been shown to be important in Th1 development, and development of Th1 cells with use of IL-12 and anti-IL-4 has previously been demonstrated (31). In this report we show that, under such conditions following a switch from Th2 to Th1 phenotype, production of IL-17 can also be induced in two of six clones. Induction of a switch from Th2 to Th1 phenotype together with the production of IL-17 further strengthens the contention that IL-17 is a Th1/Th0 cytokine. In both types of experiments, IL-17 was produced only by Th1/Th0 cells although not all Th1/Th0 cells had the capacity to produce IL-17. About 30% of our Th1/Th0 clones and two of six switched clones produced IL-17. This indicates that only a limited number of Th1/Th0 cells have the capacity to secrete IL-17. These cells could therefore represent a special subset of CD4⁺ T cells with ability to induce inflammation by use of mechanisms other than IFN-γ.

Th1 and Th0 clones were developed from three patients (NHE, SHK, and TFH), and clones producing IL-17 were found in each patient. The Th2 clones tested for IL-17 were all from the same

Table IV. *IL-17 production in relation to membrane markers expression[a]*

		CCR3		CD28		CD45RB^dim		CD57		CD27	
		+	−	+	−	+	−	+	−	+	−
IL-17	+	2[b]	3	1	4	5	0	0	5	0	5
IL-17	−	13	9	19	9	26	2	3	25	0	28
Total		15	12	20	13	31	2	3	30	0	33

[a] There was no significant association between IL-17 production and expression of the different membrane markers shown (Fischer exact test).
[b] Both of these two clones positive for CCR3 expression produced small amount of IL-4.
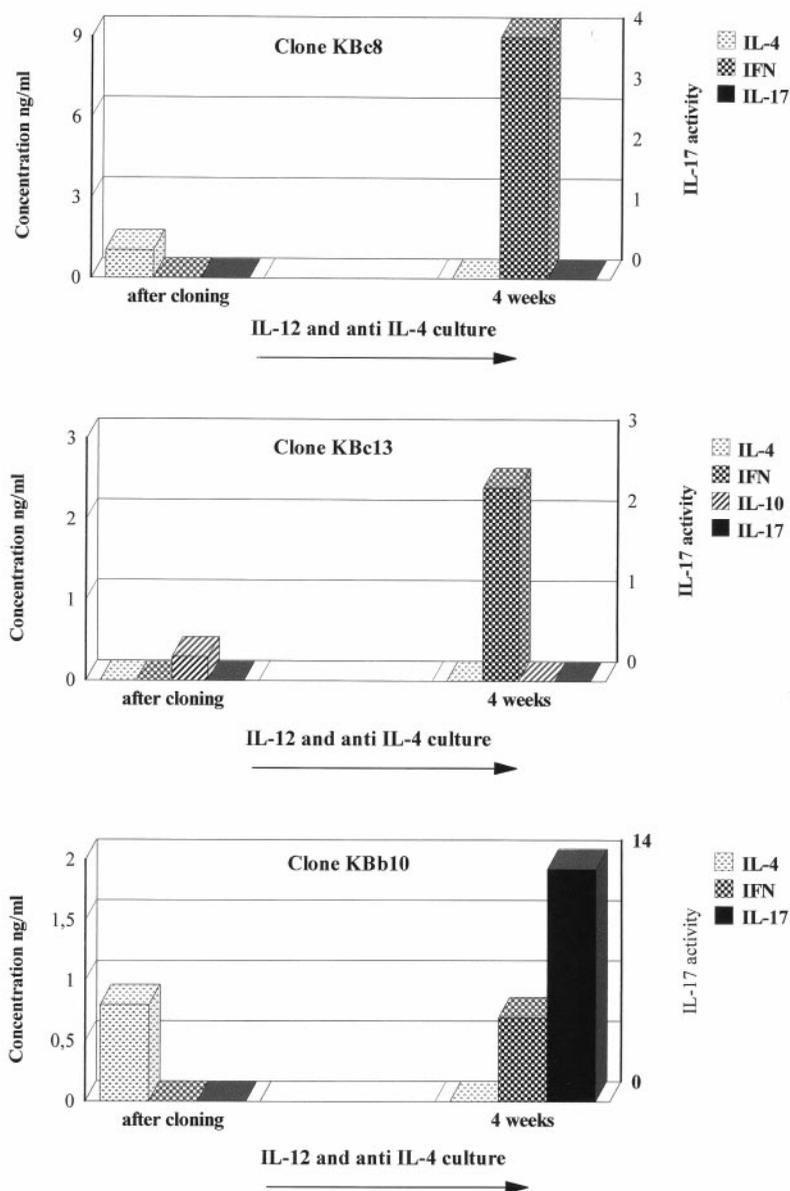
FIGURE 2. Induction of IL-17 production during Th1 development. Three Th2 clones were cultured in Th1 environment using IL-12 and anti-IL-4 for 4 wk. The clones were then stimulated with immobilized anti-CD3 for 24 h and analyzed for production of IL-17, IFN-γ, and IL-4.

patient (KB). Each of the four patients is classified as RA or JRA according to American College of Rheumatology criteria, and the Th1, Th0, and Th2 clones developed from the different patients represent typical human T cell subsets as defined by their lymphokine synthesis. We cannot exclude changes in the clones during the cloning procedure, although the cytokine comparison should be correct and representative since both IL-17 and cytokines used to determine Th1/Th2 phenotype were analyzed at the same point of time.

T cell clones were developed using the mitogen PHA and the mycobacterial Ag BCG. BCG was chosen because this Ag has been implicated in RA (32, 33). Clones raised with BCG showed reactivity against this Ag and were all of the Th1 type. This is in accordance with other findings, which have shown that mycobacterial Ags drive Th1 development (34–36). Clones raised with PHA should be representative of the total T cell population. Although IL-2 used as a growth factor for the T cell clones in these experiments can enhance IFN-γ production (37), we developed T cell clones of all the Th phenotypes: Th1, Th0, and Th2 type.

IL-10 found in three of five IL-17-positive clones is defined as a Th2 cytokine even though it has been shown that also Th1 cells can produce it (38). Moreover, IL-10 is an immunosuppressive cytokine although the level of expression may not be sufficient for down-modulation of immune activation in the joint. The clones producing IL-4 and IL-10 together with IL-17 and IFN-γ could reflect subtypes of cells that are Th1 types or Th0 cells in the beginning of their differentiation along the Th1 pathway.

In contrast to the difficulties of detecting other T cell cytokines in the RA synovium (39, 40), IL-17 can readily be detected. The low concentration of IFN-γ compared with the high levels of IL-17 could be due to competition with IL-10, since IL-10 can down-regulate IFN-γ production. Moreover, our recent studies suggest that IL-10 has no such effect on IL-17 (M. Chabaud, unpublished data). Even though the IL-17 concentration is detectable in the synovium, the numbers of IL-17-producing cells are relatively low, indicating that the contribution of IL-17 from each Th1/Th0 cell is high. This IL-17 production by some Th1/Th0 cells in the rheumatoid synovium could be a more potent way for T cells to influence inflammatory cells directly (41). Besides RA, Th1/Th0 cells producing IL-17 will probably affect other Th1-mediated chronic diseases such as multiple sclerosis, psoriasis, and diabetes, and control of such cells could have potential therapeutic antiinflammatory effects.

In conclusion, these results show a subtype of CD4$^+$ T cells associated to the Th1/Th0 phenotype that produce high levels of IL-17. This IL-17 production appears to be of importance in inflammatory reactions in RA, since its biological activity is directly related to the induction of proinflammatory cytokines found in the synovium. Further studies of the functional properties of these IL-17-producing subsets could lead to therapeutic antiinflammatory effects in Th1 mediated chronic diseases.

References

1. Fossiez, F., O. Djossou, P. Chomarat, L. Flores-Romo, S. Ait-Yahia, C. Maat, J. J. Pin, P. Garrone, E. Garcia, S. Saeland et al. 1996. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J. Exp. Med. 183:2593.*
2. 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.*
3. Mosmann, T. R., H. Cherwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol. 136:2348.*
4. Romagnani, S. 1991. Human TH1 and TH2 subsets: doubt no more. *Immunol. Today 12:256.*
5. Sallusto, F., C. R. Mackay, and A. Lanzavecchia. 1997. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. *Science 277:2005.*
6. Sallusto, F., D. Lenig, C. R. Mackay, and A. Lanzavecchia. 1998. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J. Exp. Med. 187:875.*
7. Tisch, R., and H. McDevitt. 1996. Insulin-dependent diabetes mellitus. *Cell. 85: 291.*
8. Feldmann, M., F. M. Brennan, and R. N. Maini. 1996. Role of cytokines in rheumatoid arthritis. *Annu. Rev. Immunol. 14:397.*
9. Panayi, G. S., J. S. Lanchbury, and G. H. Kingsley. 1992. The importance of the T cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis. *Arthritis Rheum. 35:729.*
10. Cush, J. J., and P. E. Lipsky. 1991. Cellular basis for rheumatoid inflammation. *Clin. Orthop. 265:9.*
11. Miossec, P., and W. van den Berg. 1997. Th1/Th2 cytokine balance in arthritis. *Arthritis Rheum. 40:2105.*
12. Adorini, l., J.-C. Guéry, and S. Trembleau. 1996. Manipulation of the Th1/Th2 cell balance: an approach to treat human autoimmune diseases? *Autoimmunity 23:53.*
13. Salmon, M., and J. S. Gaston. 1995. The role of T-lymphocytes in rheumatoid arthritis. *Br. Med. Bull. 51:332.*
14. Quayle, A. J., P. Chomarat, P. Miossec, J. Kjeldsen-Kragh, O. Forre, 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.*
15. Dolhain, R. J. E. M., A. N. van der Heiden, N. T. ter Haar, F. C. Breedveld, and A. M. M. Miltenburg. 1996. Shift toward T lymphocytes with a T helper 1 cytokine-secretion profile in the joints of patients with rheumatoid arthritis. *Arthritis Rheum. 39:1961.*
16. Miltenburg, A. M., J. M. van Laar, R. de Kuiper, M. R. Daha, and F. C. Breedveld. 1992. T cells cloned from human rheumatoid synovial membrane functionally represent the Th1 subset. *Scand. J. Immunol. 35:603.*
17. Buchan, G., K. Barrett, T. Fujita, T. Taniguchi, R. Maini, and M. Feldmann. 1988. Detection of activated T cell products in the rheumatoid joint using cDNA probes to interleukin-2 (IL-2) IL-2 receptor and IFN- gamma. *Clin. Exp. Immunol. 71:295.*
18. Firestein, G. S., and N. J. Zvaifler. 1990. How important are T cells in chronic rheumatoid synovitis? *Arthritis Rheum. 33:768.*
19. Chabaud, M., F. Fossiez, J. L. Taupin, and P. Miossec. 1998. 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. *J. Immunol. 161:409.*
20. Lotz, M., L. Bober, S. Narula, and J. Dudler. 1996. IL-17 promotes cartilage degradation. *Arthritis Rheum. 39(Suppl.):S120(Abstr.).*
21. Zvaifler, N. J. 1995. Rheumatoid arthritis: the multiple pathways to chronic synovitis. *Lab. Invest. 73:307.*
22. Alvaro-Gracia, J. M., N. J. Zvaifler, and G. S. Firestein. 1989. Cytokines in chronic inflammatory arthritis. IV. Granulocyte/macrophage colony-stimulating factor-mediated induction of class II MHC antigen on human monocytes: a possible role in rheumatoid arthritis. *J. Exp. Med. 170:865.*
23. Aarvak, T., M. Chabaud, P. Miossec, and J. B. Natvig. 1997. Analysis of IL-17 and other cytokines and surface markers of RA inflammatory T cell clones. *Arthritis Rheum. 40(Suppl.):S272(Abstr.).*
24. MacGregor A. J., and A. J. Silman. 1998. Classification and epidemiology. In *Rheumatology*. 2nd ed. J. H. Klippel and P. A. Dieppe, eds. Mosby, London, U.K., p. 2.1.
25. Kjeldsen-Kragh, J., A. Quayle, C. Kalvenes, O. Forre, D. Sorskaar, O. Vinje, J. Thoen, and J. B. Natvig. 1990. T $\gamma\delta$ cells in juvenile rheumatoid arthritis and rheumatoid arthritis: in the juvenile rheumatoid arthritis synovium the T $\gamma\delta$ cells express activation antigens and are predominantly V δ 1$^+$, and a significant proportion of these patients have elevated percentages of T $\gamma\delta$ cells. *Scand. J. Immunol. 32:651.*
26. 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.*
27. Rogge, L., L. Barberis-Maino, M. Biffi, N. Passini, D. H. Presky, U. Gubler, and F. Sinigaglia. 1997. Selective Expression of an interleukin-12 Receptor Component by human T Helper 1 Cells. *J. Exp. Med. 185:825.*
28. Aarvak, T., M. Chabaud, P. Miossec, and J. B. Natvig. 1998. Manipulation of the Th cytokine profile in CD4$^+$ T cell subsets from inflammatory rheumatoid synovium. *Arthritis Rheum. 41(Suppl.):S33(Abstr.)*
29. 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.*
30. Tortorella, C., H. Schulze-Koops, R. Thomas, J. B. Splawski, L. S. Davis, L. J. Picker, and P. E. Lipsky. 1995. Expression of CD45RB and CD27 identifies subsets of CD4$^+$ memory T cells with different capacities to induce B cell differentiation. *J. Immunol. 155:149.*
31. Szabo, S. J., A. S. Dighe, U. Gubler, and K. M. Murphy. 1997. Regulation of the interleukin (IL)-12R β2 subunit expression in developing T helper 1(Th1) and Th2 cells. *J. Exp. Med. 185:817.*
32. Quayle, A. J., K. B. Wilson, S. G. Li, J. Kjeldsen-Kragh, F. Oftung, T. Shinnick, M. Sioud, O. Forre, J. D. Capra, and J. B. Natvig. 1992. Peptide recognition, T cell receptor usage and HLA restriction elements of human heat-shock protein (hsp) 60 and mycobacterial 65-kDa hsp-reactive T cell clones from rheumatoid synovial fluid. *Eur. J. Immunol. 22:1315.*
33. Sioud, M., J. Kjeldsen-Kragh, A. J. Quayle, H.G. Wiker, D. Sorskaar, J. B. Natvig, and O. Forre. 1991. Immune responses to 18.6 and 30-kDa mycobacterial antigens in rheumatoid patients, and V β usage by specific synovial T-cell lines and fresh T cells. *Scand. J. Immunol. 34:803.*
34. Barnes, P. F., S. Lu, J. S. Abrams, E. Wang, M. Yamamura, and R. L. Modlin. 1993. Cytokine production at the site of disease in human tuberculosis. *Infect. Immun. 61:3482.*
35. Conradt, P., and S. H. Kaufmann. 1995. Impact of antigen-presenting cells on cytokine profiles of human Th clones established after stimulation with *Mycobacterium tuberculosis* antigens. *Infect. Immun. 63:2079.*
36. O'Garra, A., and K. Murphy. 1994. Role of cytokines in determining T-lymphocyte function. *Curr. Opin. Immunol. 6:458.*
37. Trinchieri, G., M. Wysocka, A. D'Andrea, M. Rengaraju, M. Aste-Amezaga, M. Kubin, N. M. Valiante, and J. Chehimi. 1992. Natural killer cell stimulatory factor (NKSF) or interleukin-12 is a key regulator of immune response and inflammation. *Prog. Growth Factor. Res. 4:355.*
38. Cohen, S. B., P. D. Katsikis, C. Q. Chu, H. Thomssen, L. M. Webb, R. N. Maini, M. Londei, and M. Feldmann. 1995. High level of interleukin-10 production by the activated T cell population within the rheumatoid synovial membrane. *Arthritis Rheum. 38:946.*
39. Simon, A. K., E. Seipelt, and J. Sieper. 1994. Divergent T-cell cytokine patterns in inflammatory arthritis. *Proc. Natl. Acad. Sci. USA 91:8562.*
40. Firestein, G. S., W. D. Xu, K. Townsend, D. Broide, J. Alvaro-Gracia, A. Glasebrook, and N. J. Zvaifler. 1988. Cytokines in chronic inflammatory arthritis. I. Failure to detect T cell lymphokines (interleukin 2 and interleukin 3) and presence of macrophage colony-stimulating factor (CSF-1) and a novel mast cell growth factor in rheumatoid synovitis. *J. Exp. Med. 168:1573.*
41. Fouilhoux, A., M. Chabaud, F. Fossiez, and P. Miossec. 1997. Production of IL-17 and its regulation in rheumatoid synovium. *Arthritis Rheum. 40(Suppl.): S272(Abstr.).*