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High Levels of IL-17 in Rheumatoid Arthritis Patients: IL-15 Triggers In Vitro IL-17 Production Via Cyclosporin A-Sensitive Mechanism

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Recent data suggest that IL-15 plays an important role in the pathogenesis of rheumatoid arthritis. In the present study, we hypothesized that elevated in the joints of rheumatoid arthritis, but not osteoarthritis, patients, IL-15 may exert its proinflammatory properties via the induction of IL-17, a cytokine known to stimulate synoviocytes to release several mediators of inflammation including IL-6, IL-8, GM-CSF and PGE₂. To test this hypothesis, we first measured the levels of IL-17 and IL-15 using specific ELISA and found that synovial fluids of patients with rheumatoid arthritis, but not with osteoarthritis, contain high levels of these cytokines. A strong correlation between IL-15 and IL-17 levels in synovial fluids was observed. Among tested factors, LPS and TNF-α failed, IL-15 and IL-2 were equipotent, and PMA + ionomycin was far more efficient in the induction of IL-17 secretion by PBMCs isolated from healthy blood donors. Interestingly, synovial fluid cells, in contrast to PBMCs isolated from patients with rheumatoid arthritis, but not osteoarthritis, respond to PMA + ionomycin with much lower, comparable to IL-15-triggered IL-17 secretion. Moreover, PMA + ionomycin-triggered IL-17 secretion is completely or partially blocked in the presence of low doses of cyclosporin A or high doses of methylprednisolone, respectively. IL-15-triggered IL-17 secretion by PBMCs was completely inhibited by these drugs. Thus, our results suggest for the first time that IL-15 may represent a physiological trigger that via cyclosporin A and steroid sensitive pathways leads to the overproduction of IL-17 in the joints of rheumatoid arthritis patients. The Journal of Immunology, 2000, 164: 2832–2838.

The soluble or membrane bound cytokines, interacting with specific receptors expressed on a variety of cell types, modulate their synthesis, secretion, and expression, thus creating a complex cytokine network that influences immunity and inflammation. Although the balance between the production of pro- and antiinflammatory cytokines and/or their functional receptors changes during the course of these processes, usually it is well self-controlled and eventually reaches the equilibrium. However, in certain situations (e.g., rheumatoid arthritis (RA)), this balance may be shifted toward the production of proinflammatory cytokines for too long, bringing more harm than help. For example, in RA patients the overproduction of TNF-α and IL-1β that contributes to the induction of other proinflammatory mediators (IL-6, IL-8, GM-CSF, and many others), seems to be critical for chronic inflammation, ultimately resulting in a joint destruction (1). Recent attempts to neutralize TNF-α (using either neutralizing Abs (2) or soluble receptors (3)) or IL-1β (using soluble IL-1R antagonist (4)) yielded promising results in controlling chronic inflammation and cartilage degradation, respectively. However, despite these encouraging results, none of these treatments cured the disease. Therefore, it is likely that cytokines or factors other than TNF-α and IL-1β also participate in the induction/expansion of proinflammatory cytokine cascade.

IL-15 was recently identified as one of these candidate mediators. The concentration of this cytokine is elevated in synovial fluids of RA patients (5–7). Interestingly, fibroblast-like synoviocytes isolated from joints of these patients spontaneously secrete large amounts of IL-15 that are further elevated on stimulation with TNF-α and IL-1β (8). After IL-15 activation, synovial T cells both secrete TNF-α directly and induce TNF-α synthesis by macrophages through cognate interactions (7). In addition, neutralization of IL-15 biological effects using either soluble IL-15R α-chain (9) or antagonist IL-15 mutant/Fcγ2a (10) proteins prevents murine collagen-induced arthritis and delayed type hypersensitivity. Based on these data, it has been proposed that IL-15 is an important player leading to/coordinates the induction of proinflammatory cascade (11, 12).

Recently, the list of potential contributors to the pathogenesis of RA has been expanded for yet another cytokine, IL-17. This cytokine is produced in present in RA synovium CD4⁺CD45RO⁺ memory T cells on activation with PMA/ionomycin or CD3/CD28 Abs (13, 14). More interesting, IL-17 triggers human synoviocytes to produce IL-6, IL-8, GM-CSF, and, of one of the major mediator of inflammation, PGE₂ (13–15). The list of biological effects of IL-17 includes also the stimulation of granulopoiesis (16), and osteoclastogenesis (17), the up-regulation of NO synthesis in cultured human cartilage (18), and triggering of the production of TNF-α, IL-1β, IL-12, interleukin-6, IL-10, and IL-1R antagonist in human peripheral blood macrophages (19).
In the present report, we hypothesized that elevated in RA, but not in osteoarthritis (OA), synovial IL-15 triggers IL-17 production that, in turn, exerts its biological activity stimulating the production of other proinflammatory mediators. To test this hypothesis we have compared: 1) the levels of IL-17 and IL-15 in serum and synovial fluids of RA and OA patients; and 2) IL-15-triggered in vitro production of IL-17 by PBMCs and synovial fluid mononuclear cells (SFMCs) isolated from these patients. In addition, in attempts to block triggered in vitro IL-17 production, we have applied known immunosuppressive drugs: cyclosporin A (CsA) and methylprednisolone (MP).

We report high (RA) and undetectable (OA) levels of IL-17 that strongly correlate with the levels of IL-15 in synovial fluids of these patients. We show for the first time that 1) IL-15, but not TNF-α, stimulates IL-17 production by PBMCs in primary culture and 2) CsA completely and MP partially block PMA/ionomycin-triggered IL-17 production, whereas IL-15-induced IL-17 production is more sensitive to MP and slightly less sensitive to CsA. Thus, our data stress the important role of IL-15 as the inductor of other proinflammatory cytokines (in our report IL-17). Moreover, it is possible that blockade of IL-17 production, if occurs in vivo at least partially, at good results achieved using CsA in the treatment of RA (20–23).

**Patients and Methods**

**Reagents**

Recombinant human IL-15, IL-17, and anti-IL-17 IgG1 mAb (M68) were kindly provided by Dr. T. Troutt (Immunex, Seattle, WA). The following Abs and cytokines were used: recombinant human TNF-α, IL-2, IL-1β, IL-6, IL-8, normal mouse IgG1 (mAb), normal goat IgG, protein G-Sepharose-purified polyclonal goat IgG, anti-TNF-α, and anti-IL-15 from R&D Systems (Minneapolis, MN); polyclonal rabbit anti-IL-17 from Chemicon (Temecula, CA); polyclonal rabbit anti-IL-15 from PeproTech (Rocky Hill, NJ); polyclonal rabbit anti-TNF-α, mouse anti-IL-15 mAb (M111), and recombinant human IL-15 from Genzyme (Cambridge, MA). Peroxidase-conjugated goat anti-rabbit IgG, α-phenylenediamine, PMA, PHA-P, ionomycin, and hyaluronidase (type I-S) were obtained from Sigma (St. Louis, MO). MaxiSorp and 24-well plates were purchased from Nunc (Roskilde, Denmark). The following immunosuppressive drugs were used in this study: Solu-Medrol (MP, Upjohn, Paris, France) and Sandimmun (cyclosporin A, Novartis, Bern, Switzerland).

**Patients and control subjects**

Paired peripheral blood and synovial fluids were obtained from 15 patients with RA and 8 patients with OA. Blood samples were also obtained from 20 healthy individuals. The patients fulfilled the revised criteria of the American College of Rheumatology for RA (24) or for OA (25). The mean age of RA patients was 51 years (range, 32–76 years), and the mean duration of disease was 75 mo (range, 4–180 mo). The mean ± SD erythrocyte sedimentation rate was 56 ± 39. Nine RA patients were seropositive for IgM rheumatoid factor. Eleven RA patients were receiving prednisolone at low dose, and 4 patients disease-modifying antirheumatic drugs. The mean age of OA patients was 46 years (range, 23–80 years), and the mean duration of disease was 39 mo (range, 3–120 mo). The mean ± SD erythrocyte sedimentation rate was 37 ± 50. All patients with OA received only nonsteroidal antiinflammatory drugs.

**Cells and culture conditions**

Mononuclear cells were isolated by density gradient centrifugation of heparinized blood or synovial fluids using GradiSol L (Biomed, Lublin, Poland). Cells (2 × 10⁶/ml) were cultured in 24-well plates in RPMI 1640 supplemented with 2 mM l-glutamine, 10% FCS (Seromed, Berlin, Germany), 100 U/ml penicillin, 100 µg/ml streptomycin, and 1 mM HEPES at 37°C in an atmosphere of 95% air and 5% CO₂. Cells were stimulated for 72 h in the presence or absence of a mixture of PMA (1 nM) and ionomycin (3 µg/ml), or LPS (5 µg/ml) (Escherichia coli 055:B5; Difco, Detroit, MI), or PHA (5 µg/ml), or cytokines: IL-15 (0, 1–300 ng/ml; Immunex); IL-2 (0, 1–300 ng/ml); TNF-α (0, 1–10 ng/ml); IL-6 (10 ng/ml); IL-1β (10 ng/ml); and IL-8 (10 ng/ml). To study the effect of neutralizing anti-IL-15 mAb on the IL-15-induced production of IL-17, the recombinant human IL-15 (100 ng) was incubated with or without anti-IL-15 mAb (M111, 3 µg) or normal mouse mAb (3 µg) for 30 min at 37°C before addition to the cell cultures. CsA (0.005–400 ng/ml) or MP (0.004–20 µg/ml) were added 1 h before activators. After incubation, the culture supernatants were collected and clarified by centrifugation at 400 × g for 10 min, and the concentrations of IL-17 and TNF-α were determined by ELISA.

**Immunoassays for IL-17, IL-15, and TNF-α**

The concentrations of IL-17, IL-15, and TNF-α were measured by a sandwich ELISA using anti-IL-17 mAb (M68), or polyclonal goat IgG anti-IL-15, or polyclonal goat IgG anti-TNF-α, as coating Abs, respectively. To eliminate interference caused by heterophile Abs, the control wells were coated with normal mouse IgG1 (ELISA for IL-17) or normal goat IgG (ELISA for IL-15) and were applied as a blank for individual sera and synovial fluids. Next, the polyclonal rabbit anti-IL-17, anti-IL-15, or anti-TNF-α was used as a detecting Ab, followed by peroxidase-conjugated goat anti-rabbit IgG. Recombinant human IL-17, IL-15 (Genzyme, Cambridge, MA), and TNF-α were used as standards. The peroxidase reaction was developed using α-phenylenediamine dihydrochloride as a substrate. The optical density was measured at 492 nm with an automatic ELISA reader (LP 400, Diagnostic Pasteur, Marnes la Coquette, France). The detection limit was 15 pg/ml for IL-17 and IL-15 or 4 pg/ml for TNF-α.

Synovial fluids were collected into the tubes with heparin, and the cells were removed by centrifugation (400 × g, 10 min). Before assay, each synovial fluid was treated with hyaluronidase at a concentration of 10 U/ml for 80 min at 37°C followed by centrifugation (12,000 × g, 5 min). This treatment was performed to reduce the viscosity of synovial fluid. To exclude the possible interference of rheumatoid factor in the immunoassays, the sera and synovial fluid were preadsorbed on human γ-globulin-coated polystyrene beads (Biomed). Moreover, serum and synovial fluid samples were diluted twice in PBS containing 0.5% BSA, 0.05%Tween 20, and 1 µg/ml normal mouse IgG1 (ELISA for IL-17) or normal goat IgG (ELISA for IL-15).

**Statistical analysis**

Data are expressed as the mean ± SEM. The two-tailed Mann-Whitney U test was used to compare the levels of cytokines in different groups of patients. The paired samples were analyzed by paired two-tailed t test. Correlation between IL-17 and IL-15 levels was assessed with Pearson’s

![FIGURE 1](http://www.jimmunol.org/Downloaded-from)
correlation coefficient. Probability values <0.05 were considered statistically significant.

Results

Synovial fluids of RA, but not OA, contain high levels of IL-17 and IL-15

To measure IL-17 and IL-15 levels, we used highly specific ELISAs according to protocols described in Patients and Methods. To eliminate possible interference with rheumatoid factor or heterophilic Abs, all sera and synovial fluids were: 1) preadsorbed on human γ-globulin-coated polystyrene beads; and 2) diluted twice with buffer containing normal IgG from the same species as coated Abs used in ELISA. In addition, for each serum and synovial fluid, individual blank wells were prepared. In these wells, capture anti-IL-17 (or anti-IL-15) Abs were substituted with preimmune IgG. The OD values of these wells were considered as the real blank values and therefore subtracted from the OD values of other wells. We believe that these precautions prevent the possible interference with rheumatoid factors in ELISAs for IL-15 and IL-17. Using these pretreatments and ELISAs, we observed elevated concentrations of IL-17 and IL-15 in synovial fluids of RA in comparison to OA patients (Fig. 1). Moreover, we observed strong correlation between IL-15 and IL-17 levels in the synovial fluids of these patients (Table I). On the other hand, there was no correlation between levels of IL-17 in synovial fluids and serum of the same patients. Finally, we show that elevated levels of IL-15 were also present in the serum of RA but not OA patients (Fig. 1). In contrast to IL-17, the levels of IL-15 present in serum and synovial fluids of the same patients were correlated (Table I).

IL-15, IL-2, and PHA trigger moderate, whereas PMA + ionomycin induce high secretion/production of IL-17 by PBMCs from healthy blood donors

The main cell type producing IL-17 has been identified as a CD4+CD45RO+ memory T cell (14). Although cells of this phenotype are abundant in joints of RA patients (26), the physiological trigger of IL-17 production and/or its release remains unknown. We hypothesized that IL-15, also elevated in the joints of RA patients (5, 27) and which may originate from macrophage- and fibroblast-like synoviocytes (8), participated in the induction of IL-17 production. Indeed, in in vitro experiments, IL-15 in a dose-dependent manner triggered IL-17 production/secretion by mononuclear cells isolated from healthy blood donors (Fig. 2). Anti-IL-15 Abs inhibit this effect, confirming that indeed IL-15 is responsible for the observed rise in IL-17 production in our culture system (Fig. 3). Among tested stimuli, LPS failed; IL-15, IL-2, and PHA triggered moderate; and PMA + ionomycin induced high production of IL-17 (Table II). In addition, exogenous addition of TNF-α, IL-8, or IL-6 also failed to trigger IL-17 production by PBMCs, whereas IL-1β was roughly 30% less potent than IL-15 (Fig. 4). The mixture of TNF-α, IL-6, IL-8 with IL-1β, or all these cytokines with IL-15 did not influence the IL-1β- or IL-15-triggered IL-17 production, respectively (Fig. 4). Interestingly, even after 72 h of the culture, there was no detectable level of spontaneously released IL-17. We concluded that IL-15, IL-2, and to a lesser extend IL-1β represent physiological factors that trigger IL-17 production/release.

Production of IL-17 by mononuclear cells isolated from blood and synovial fluids of RA and OA patients

PBMCs and SFMCs isolated from patients with RA or OA, similar to PBMCs from healthy blood donors, were also triggered by IL-15 to produce IL-17 (Fig. 5). However, higher levels of IL-15-triggered IL-17 secretion (although not statistically significant) were observed in supernatants of cells isolated from synovial fluids than from peripheral blood of RA patients (Fig. 5B). Cells isolated from synovial fluid and PB of OA patients did not differ in their response to IL-15 stimuli (Fig. 5B). Surprisingly, SFMCs from RA, but not from OA, patients exerted impaired response to PMA + ionomycin-triggered production of IL-17 (Fig. 5A), whereas PBMCs from the same patients responded as well as cells isolated from healthy blood donors. Taken together, these results, showing that synovial fluid cells are significantly different from PBMCs in their ability to synthesize IL-17 in response to different stimuli, suggest preferential exclusion of cells capable to respond fully to PMA + ionomycin from RA synovial fluid. In addition, although

![FIGURE 2. IL-15 triggers IL-17 production by PBMCs from healthy individuals. PBMCs were stimulated for 72 h with increasing concentrations of IL-15. The levels of IL-17 were measured in culture supernatants by ELISA. Data are expressed as the means ± SEM. One representative result of four independent experiments is shown.](http://www.jimmunol.org/)

![FIGURE 3. IL-15-induced production of IL-17 is inhibited in the presence of neutralizing anti-IL-15 mAb. PBMCs were stimulated for 72 h with or without IL-15 (100 ng/ml) and anti-IL-15 mAb (M111; 3 μg/ml) or control mouse mAb (3 μg/ml). Data are expressed as the means ± SEM. One representative experiment of two that produced similar results is shown. * p < 0.033 compared with IL-15 (paired two-tailed t test).](http://www.jimmunol.org/)

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<table>
<thead>
<tr>
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<th>Parameter B</th>
<th>r</th>
<th>p</th>
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<tr>
<td>Serum IL-15</td>
<td>SF IL-15</td>
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<td>&lt;0.02</td>
</tr>
<tr>
<td>Serum IL-17</td>
<td>SF IL-17</td>
<td>0.299</td>
<td>NS</td>
</tr>
<tr>
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<td>Serum IL-17</td>
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<td>&lt;0.05</td>
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<td>SF IL-17</td>
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<td>SF IL-15</td>
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<td>NS</td>
</tr>
<tr>
<td>Serum IL-15</td>
<td>SF IL-17</td>
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<td>&lt;0.02</td>
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not significant in our limited studies, there is a tendency for positive selection of IL-15-responsive cells into the synovial fluid.

**CsA and MP inhibit IL-17 production by PBMCs from healthy blood donors**

Next, we tested the sensitivity of IL-17 production to the known immunosuppressive drugs CsA and MP. The PMA/ionomycin-triggered IL-17 production by healthy blood donors PBMCs was entirely blocked in the presence of CsA at concentrations as low as 50 ng/ml (Fig. 6A). Interestingly, the production of TNF-α, measured in the same supernatants, was inhibited by not more than 80%, even when high concentrations of CsA (400 ng/ml) were applied (Fig. 6A). In contrast, MP, even at very high concentrations (20 µg/ml) blocked IL-17 and TNF-α production by only 40 and 60%, respectively (Fig. 6B). In contrast, the sensitivity of IL-15-triggered IL-17 production to these drugs was different; lower concentrations of MP and slightly higher of concentrations of CsA were required for complete inhibition of this cytokine production (Fig. 7). These results, showing that like several other cytokines (e.g., IL-2, IL-4) the production of IL-17 is entirely blocked in the presence of CsA, suggest that activation of calcineurin and further downstream dephosphorylation of NF-AT and its translocation to the nucleus play a pivotal role in the induction of IL-17 transcription.

**Discussion**

In this paper, we report elevated levels of IL-17 in synovial fluids of RA, but not OA, patients (Fig. 1). Since submission of the first version of the paper, the elevation of IL-17 in synovial fluids of RA and lack of this cytokine in patients with OA have been reported (17). Based on our current knowledge, CD4⁺ CD45RO⁺ memory T cells are a major T cell subset present in the synovium of RA patients (26). The same subset of T cells produces large amounts of IL-17 on stimulation (14). Moreover, in synovial tissue of RA patients, these cells are immunoreactive with anti-IL-17 Abs (17). In contrast, a low level of IL-17 has been produced by CD8⁺ T cells (13), and CD8⁺ cells present in synovial tissue isolated from RA patients fail to immunoreact with anti-IL-17 Abs (17). In addition, it has been shown that cultured pieces of RA synovium (containing CD4⁺ CD45RO⁺ cells) also produce IL-17 (28, 29). Thus, it is very likely that also in vivo CD4⁺ CD45RO⁺ T cells represent the main source of IL-17. There is one report indicating that only 1% of T cells present in synovial tissue are IL-17 producers. Further studies are required if the same low proportion of IL-17-producing cells is preserved in synovial fluid.

Also, we report for the first time that IL-17 production is induced by IL-15. The significance of this finding is underlined by recent evidence that IL-15 is one of the most critical factors contributing to the pathogenesis of RA (11). Indeed, high levels of IL-15 in synovium (5, 27) and according to our finding in serum of RA but not OA patients (Fig. 1) are present. In addition, IL-15 has been shown to trigger the production of other proinflammatory cytokines including TNF-α (7, 30), IL-8, monocyte chemotactic protein 1 (31), IL-12, and IFN-γ (32). On the other hand, a recent report indicates that TNF-α and IL-1β may also stimulate IL-15 production by human synoviocytes (8), completing the loop of self-perpetuating proinflammatory cytokine synthesis. Although we fail to detect IL-17 production after addition of TNF-α, IL-6, or IL-8 (Fig. 4), the low level of IL-17 produced in response to IL-1β

### Table II. Secretion of IL-17 and TNF-α by PBMCs from healthy individuals

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Control</th>
<th>LPS (5 µg/ml)</th>
<th>IL-15 (100 ng/ml)</th>
<th>IL-2 (100 ng/ml)</th>
<th>PHA (5 µg/ml)</th>
<th>PMA + Ionomycin (1 nM + 3 µg/ml)</th>
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<tr>
<td>IL-17 (pg/ml)</td>
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<td>9</td>
<td>144</td>
<td>157</td>
<td>254</td>
<td>150,000</td>
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<tr>
<td>Mean ± SEM</td>
<td>1 ± 1</td>
<td>14 ± 8</td>
<td>164 ± 35</td>
<td>138 ± 54</td>
<td>563 ± 175</td>
<td>231,691 ± 55,749</td>
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<tr>
<td>TNF-α (pg/ml)</td>
<td>33</td>
<td>500</td>
<td>225</td>
<td>111</td>
<td>223</td>
<td>13,080</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>38 ± 12</td>
<td>515 ± 37</td>
<td>243 ± 61</td>
<td>378 ± 280</td>
<td>322 ± 80</td>
<td>11,984 ± 2,261</td>
</tr>
</tbody>
</table>

*PBMCs from 14 healthy individuals were stimulated for 72 h with the indicated concentrations of activators. IL-17 and TNF-α secretion was determined in culture supernatants by ELISA. ND, not detectable.*

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**FIGURE 4.** Production of IL-17 by PBMCs from healthy individuals. PBMCs from healthy individuals were stimulated for 72 h with TNF-α (10 ng/ml), IL-8 (10 ng/ml), IL-6 (10 ng/ml), IL-1β (10 ng/ml), IL-15 (100 ng/ml), or the mixture containing all of the above cytokines (10 ng/ml of each) with and without IL-15 (100 ng/ml). The levels of IL-17 were measured in culture supernatants by ELISA. Each value represents the mean ± SEM of three independent experiments. *, p < 0.047 and **, p < 0.007 vs control (paired two-tailed t test).

**FIGURE 5.** Production of IL-17 by mononuclear cells from blood and synovial fluids of RA and OA patients. PBMCs and SFMCs from RA (n = 8) and OA (n = 4) patients were stimulated for 72 h with PMA (1 nM) + ionomycin (I) (3 µg/ml) (A) or IL-15 (100 ng/ml) (B). The levels of IL-17 were measured in culture supernatants by ELISA. Data are expressed as the means ± SEM. *, p < 0.023 (paired two-tailed t test).
may indicate that the latter cytokine induces IL-15, which in turn stimulates IL-17 production. Lack of any additive effect on IL-17 production when both IL-15 and IL-1β are present in the culture medium (Fig. 4) supports this notion. Nevertheless, a direct or indirect effect of IL-1β, the induction of IL-17 synthesis in response to IL-15, seems to be a logical consequence of the central role that the latter cytokine plays in the unbalanced production of proinflammatory cytokines that accelerate/maintain chronic inflammation in general, and RA in particular. Known biological properties of IL-17, as a cytokine inducing other cytokines (33) and a stimulator of osteoclastogenesis (17), fit very well to the pattern of cytokines present in RA synovium and changes in bone resorption (1) and therefore may occur in vivo. Yeh strong correlation between IL-15 and IL-17 levels in synovial fluids of RA patients (Table I) supports our hypothesis that IL-15 plays an important role in the regulation of IL-17 synthesis in vivo. On the other hand, lack of correlation between levels of IL-17 in synovial fluids and serum of the same patients (Table I) suggest that IL-17 is produced mainly, if not exclusively, locally in the synovium of RA patients. At present, it is not clear why there is no correlation between levels of IL-15 and IL-17 in the serum of these patients. One possibility would stress that IL-17-producing CD4+CD45RO+ cells are less abundant in the periphery than in the joints of RA patients and therefore that IL-15 triggers only low levels of IL-17 in the circulation. The other possibility is that serum contains inhibitor(s) of biological activity of IL-15. This hypothesis gained recently some momentum; preliminary results of Gracie et al. (34) show that, present more frequently in serum than in synovial fluid of RA patients, soluble IL-15R α-chain may block the biological activity of IL-15. In addition to IL-15, we found that also IL-2 triggers the production of IL-17 (Table II). However, in contrast to IL-15, IL-2 is hardly present in the synovium or serum of RA patients (35). Thus, abundant in RA joints, IL-15, recently shown also to be produced by fibroblast-like synoviocytes on stimulation with TNF-α and IL-1β and spontaneously by synovial macrophages (8), may be responsible for triggering present in affected joints CD4+CD45RO+ memory T cells to produce high levels of IL-17. Interestingly, our experiments show that only IL-1β, but not TNF-α, stimulates IL-17 production. Although it is not proved yet, we favor the hypothesis that in our experimental conditions, IL-1 may trigger IL-15 production which in turn stimulates T-cell to produce IL-17. This hypothesis is supported by the lack of additive effects of IL-1β and IL-15 in promoting IL-17 synthesis (Fig. 4). Taken together, the above described scenario adds a new aspect of the role of CD4+ memory cells in the pathogenesis of RA (36).

Opposite responses of cells isolated from matched samples of peripheral blood and synovial fluids from RA patients to IL-15-triggered IL-17 production suggest that 1) IL-17-producing cells isolated from the synovial fluids of these patients are more sensitive to the IL-15 trigger (e.g., exerting more IL-15 receptors) and that 2) there is a higher percentage of CD4+CD45RO+ memory T cells capable of producing IL-17 in RA synovium than in the peripheral blood. However, different responses of the same cells stimulated with PMA/ionomycin, where IL-17 production was significantly higher in mononuclear cells isolated from peripheral blood (PBMCs) than from synovial fluid (SFMCs) of RA patients (Fig. 5A), indicate that although among PBMCs are cells able to produce IL-17 in high quantities, these cells do not enter the synovium. In addition, it is possible that IL-15- and PMA + ionomycin- triggered signals that lead to IL-17 production differ significantly. This hypothesis is supported by different levels of IL-17 triggered by these stimuli. Experiments designed to sort out these complexities are currently tested in our laboratory.

Although, according to our findings, IL-17 lies downstream of IL-15, controlling the synthesis of IL-17, and therefore inhibiting even further downstream events of its overproduction, may be of vital interest of patients with chronic inflammation. This theme led us to the finding that CsA and, to a lesser extent, MP inhibit PMA + ionomycin-triggered IL-17 production in vitro (Fig. 6). Both these drugs completely blocked IL-15-triggered IL-17 production at lower (MP) and slightly higher (CsA) concentrations (Fig. 7). These differential effects on the two types of IL-17 induction may be related to the 1000-fold difference in production levels.

CsA exerts its immunosuppressive activity by blocking enzymatic activity of serine/threonine phosphatase calcineurin that is

FIGURE 6. Effects of CsA or MP on PMA + ionomycin-triggered IL-17 and TNF-α production by PBMCs from healthy individuals. PBMCs pretreated for 1 h with the indicated concentrations of CsA (A) or MP (B) were stimulated for an additional 72 h with PMA (1 nM) + ionomycin (3 μg/ml). The production of IL-17 and TNF-α were determined in culture supernatants by ELISA. Data are expressed as the means ± SEM of six experiments.

FIGURE 7. Effects of CsA or MP on IL-15-triggered IL-17 production by PBMCs from healthy individuals. PBMCs pretreated for 1 h with the indicated concentrations of CsA or MP were stimulated for an additional 72 h with IL-15 (100 ng/ml). The production of IL-17 was determined in culture supernatants by ELISA. Data are expressed as the means ± SEM of four experiments.
required for dephosphorylation of present in the cytosol-phosphorylated transcription factor NF-AT (37). Failure to dephosphorylate NF-AT prevents its translocation to the nucleus and results in lack of the initiation of transcription of several cytokines (e.g., IL-2, IL-3, IL-4, IFN-γ) (37). Of four known isoforms of NF-AT, i.e., NF-AT-p, NF-AT-c, NF-AT-3, and NF-AT-4, additive or even synergistic effects of NF-AT-p and NF-AT-4 activation are critical for the development of Th1-type immune response (38). Because IL-17 is classified as Th1 cytokine (39), it is likely that NF-AT-p and NF-AT-4 are required for the induction of its synthesis. However, the experiments testing this suggestion have to be done.

The use of CsA in RA therapy has been recently investigated, and good results were observed with relatively low doses of this drug (2–5 mg/kg/daily) (20–23). Consequently, these doses are currently recommended for RA treatment. Low concentrations of CsA that effectively block IL-17 production in vitro are even below the range of concentrations of this drug observed in patients with RA (22, 23). Therefore, it is likely that in vivo situation, the concentration of CsA, although low, is still high enough to prevent the induction of IL-17 production. The latter suggestion is currently tested in our Institute.

Our studies show also the comparison of CsA and MP in their ability to block PMA/ionomycin-induced production of IL-17 and TNF-α (Fig. 6). In these in vitro experiments, CsA is far more effective in blocking both IL-17 and, to a lesser extent, TNF-α than MP which even at relatively high concentrations inhibits only ~50% of cytokine production. Although these results are preliminary, we should have them in mind while considering therapeutic intervention into the cytokine network blocking the effects of either TNF-α (2, 3) or IL-1β (4). It is possible that combination of certain immunosuppressive drugs (e.g., CsA) with new biological agents may prove very effective.

In conclusion, we report high (RA) and undetectable (OA) levels of IL-17 that strongly correlate with the levels of IL-15 in synovial fluids of these patients. Moreover, our finding that IL-17 induces IL-17 production in vitro in the cytokine network/ caspase. Finally, we document high efficacy of CsA and MP in blocking IL-17-triggered IL-17 production in vitro. In addition, CsA is extremely, whereas MP is only partially effective in blocking PMA/ionomycin-triggered IL-17 production. Thus, if these in vitro observations apply to the situations in vivo, blocking IL-17 may represent yet another reason for using CsA in controlling unbalanced cytokine network.

References


