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IL-16 as an Anti-Inflammatory Cytokine in Rheumatoid Synovitis

Piotr A. Klimiuk, Jörg J. Goronzy, and Cornelia M. Weyand

T lymphocytes are a major component of the inflammatory infiltrate in rheumatoid synovitis, but their exact role in the disease process is not understood. Functional activities of synovial T cells were examined by adoptive transfer experiments in human synovium-SCID mouse chimeras. Adoptive transfer of tissue-derived autologous CD8+ T cells induced a marked reduction in the activity of lesional T cells and macrophages. Injection of CD8+, but not CD4+, T cells decreased the production of tissue IFN-γ, IL-1β, and TNF-α by >90%. The down-regulatory effect of adoptively transferred CD8+ T cells was not associated with depletion of synovial CD3+ T cells or synovial CD68+ macrophages, and it could be blocked by Abs against IL-16, a CD8+ T cell-derived cytokine. In the synovial tissue, CD8+ T cells were the major source of IL-16, a natural ligand of the CD4 molecule that can anergize CD4-expressing cells. The anti-inflammatory activity of IL-16 in rheumatoid synovitis was confirmed by treating synovium-SCID mouse chimeras with IL-16. Therapy for 14 days with recombinant human IL-16 significantly inhibited the production of IFN-γ, IL-1β, and TNF-α in the synovium. We propose that tissue-infiltrating CD8+ T cells in rheumatoid synovitis have anti-inflammatory activity that is at least partially mediated by the release of IL-16. Spontaneous production of IL-16 in synovial lesions impairs the functional activity of CD4+ T cells but is insufficient to completely abrogate their stimulation. Supplemental therapy with IL-16 may be a novel and effective treatment for rheumatoid arthritis. The Journal of Immunology, 1999, 162: 4293–4299.

In rheumatoid arthritis (RA), the synovial membrane of affected joints undergoes a series of changes that ultimately lead to the formation of a tissue with proliferative and aggressive character (1). Lymphocytes and macrophages are recruited into the sublining layers of the synovium. Tissue infiltration is a highly regulated process, as evidenced by a defined compartmentalization of infiltrating cells and the formation of specialized lymphoid organizations (2). Specifically, synovial lymphocytes are often arranged in follicles that resemble germinal centers in lymph nodes (3). The emergence of ectopic lymphoid tissue in a primarily nonlymphoid organ has been cited as evidence for a role of immune mechanisms in the pathogenesis of RA. In the current paradigm, RA represents the sequela of a persistent immune response to as-yet-unidentified Ags of either endogenous or exogenous origin (4, 5).

Although the topography of the inflammatory infiltrates in rheumatoid synovium with the formation of germinal centers can only be explained by a T cell-driven immune response, evidence for activation and proliferation of lesional T cells is sparse. Tissue-infiltrating T cells in rheumatoid synovitis do not display functional activities expected to occur in an Ag-driven immune response. Specifically, T cell-derived cytokines are distinctly low (6, 7). The coexistence of germinal centers with functionally subdued T cells raises the question whether mechanisms are in place that down-regulate T cell activity in the lesions. Subduced T cell responses are not related to the production of cytokines generally considered as anti-inflammatory. IL-4 cannot be detected in many synovial tissues (8). Paradoxically, the highest production of IL-4 is present in synovitis with granuloma formation (9). IL-10 is produced in the rheumatoid synovium (10), but its production correlates with the presence of germinal centers, suggesting no down-regulatory function in synovial inflammation (9).

To address the hypothesis that T cell down-regulatory mechanisms exist in the synovial infiltrate, we have studied the function of tissue-infiltrating lymphocytes in an in vivo model of human rheumatoid synovitis. By engrafting inflamed rheumatoid synovium into nonobese diabetic (NOD)-SCID mice and adoptively transferring T cell subsets, the contribution of cell types to the inflammation could be examined. Adoptive transfer of synovial tissue-derived syngeneic CD8+ T cells induced a marked reduction in T cell and macrophage activity that was blocked by Abs against the CD8+ T cell-derived cytokine, IL-16, a natural ligand of the CD4 molecule that can anergize CD4-expressing cells (11–14). The efficiency of recombinant human IL-16 (rhIL-16) was explored in a treatment trial in human synovium-SCID mouse chimeras. Daily injections of rhIL-16 significantly reduced tissue cytokine production, suggesting an immunosuppressive function of IL-16 in RA.

Materials and Methods

Study population

Synovial tissue was obtained from 11 patients who fulfilled the American College of Rheumatology 1987 criteria for RA and who were undergoing arthroplasty or synovectomy (15). All patients had active synovitis at the time of tissue collection, and histology of the synovial tissue showed dense mononuclear infiltrates. Seven patients had rheumatoid factor. Four had been treated with only nonsteroidal anti-inflammatory drugs before surgery, and the remaining seven were on low-dose corticosteroids (n = 4), hydroxychloroquine (n = 2), and/or methotrexate (n = 3). All patients were typed for their HLA-DBR1 alleles by PCR and subsequent oligonucleotide hybridization (Biotest Diagnostics, Danville, NJ). Eight patients...
expressed a disease-associated HLA-DRB1*04 allele, and two additional patients typed HLA-DRB1*01.

**Generation of human synovium-SCID mouse chimeras**

NOD/LtSz-Prkdcsd/l mice (NOD-SCID, 6–8 wk of age, The Jackson Laboratory, Bar Harbor, ME) were anesthetized with 50 mg/kg pentobarbital and methoxyflurane. Synovial tissue samples were implanted s.c. on the dorsal midline. Engraftment generally occurred within 7 days. Synovial tissue was retrieved and embedded in OCT compound (Tissue-Tek, Sakura Finetek, Torrance, CA) or shock frozen in liquid nitrogen.

**Generation of synovial T cell lines and adoptive transfer experiments**

Small pieces of synovial tissue were cultured in 24-well cell culture plates (Costar, Cambridge, MA) in RPMI 1640 supplemented with 10% FBS (Summit Biotechnology, Fort Collins, CO) and 20 IU/ml of rhIL-2 (Cellus, Emeryville, CA). Established T cell lines were maintained by weekly polyclonal restimulation and addition of 20 IU/ml rhIL-2. FACS analysis showed that T cell lines were composed of 11–56% CD4⁺ T cells and 39–85% CD8⁺ T cells. CD4⁺ and CD8⁺ T cell lines were established from synovial tissue-derived T cells by sorting for CD3⁺ CD4⁺ or CD3⁺ CD8⁺ cells on a FACSVantage (Becton Dickinson Immunocytometry Systems, San Jose, CA).

In adoptive transfer experiments, mice were implanted with synovial tissue and 14 days later were injected i.p. with 5 x 10⁷ unsorted T cells, 2.5 x 10⁷ purified CD4⁺ T cells, or 2.5 x 10⁷ purified CD8⁺ T cells expanded from nonimplanted autologous synovial tissue. In selected experiments, autologous T cells were adoptively transferred into human synovium-SCID mouse chimeras 14 days after implantation, and 250 μg of rabbit anti-human IL-16 or normal rabbit IgG (both from PeproTech, Rocky Hill, NJ) was injected i.p. on days 17 and 18 following implantation. The synovial tissues were harvested on day 22.

**Treatment with rhIL-16**

Human synovium-SCID mouse chimeras were treated with daily i.p. injections of buffer control or rhIL-16 (PeproTech) starting on day 7 after tissue implantation. Two doses of IL-16, 500 ng and 1000 ng per injection, and two treatment durations, 10 and 14 days, were tested. Implanted tissues were harvested on days 17 and 21, respectively, and were analyzed by immunohistochemistry. In situ cytokine transcription was determined by PCR-ELISA.

**Cytokine measurement by PCR-ELISA**

Total RNA was extracted from synovial tissue with Trizol (Life Technologies, Grand Island, NY), and cDNA was synthesized using oligo(dT) and avian myeloblastosis virus (AMV) reverse transcriptase (Boehringer Mannheim, Indianapolis, IN). cDNA from synovial tissue specimens was adjusted to contain equal numbers of copies (p < 0.03) of IFN-γ mRNA to 10% of control levels (Fig. 1A). The transferred cells not only inhibited T cell function, they also affected IL-1β and TNF-α production. Median tissue concentrations of IL-1β were reduced from 2,974 to 304 transcripts (p = 0.03), and TNF-α-specific sequences decreased from 17,301 to 1,454 copies (p = 0.03).

Lines were sorted into CD4⁺ and CD8⁺ T cells, and cells from each subset were adoptively transferred (Fig. 1B). Following the injection of 2.5 x 10⁷ autologous CD4⁺ T cells, the synthesis of IFN-γ and TNF-α mRNA in the tissue was essentially unchanged. Conversely, transfer of 2.5 x 10⁷ autologous synovial CD8⁺ T cells induced profound inhibition of IFN-γ (p = 0.04) as well as TNF-α transcription (p = 0.005).

Immunohistochemical studies of tissues retrieved from the chimeras demonstrated that the cellularity of the infiltrate was not influenced by the adoptively transferred T cells (Figs. 2 and 3). Tissue sections were stained with anti-CD3 and anti-CD68 mAb, and the numbers of T cells and macrophages per section were counted. The results shown in Fig. 3 are representative of three experiments. Sixty to 70 CD3⁺ T cells and 50–70 CD68⁺ macrophages per high-power field were present in the control tissues. After adoptive transfer of unseparated synovial T cells or sorted CD4⁺ or CD8⁺ T cells, the number of tissue-infiltrating CD3⁺ T cells and CD68⁺ macrophages did not change. However, the number of IFN-γ-producing and TNF-α-producing cells decreased by ~70% after transferring CD8⁺ T cells. These experiments demonstrated that synovial CD8⁺ T cells have down-regulatory activity in rheumatoid synovitis. Inhibition of T cell and macrophage function was not associated with cell depletion from the synovial lesions, suggesting that the effect was not related to a cytotoxic activity of CD8⁺ T cells.

**Expression of anti-inflammatory cytokines in synovial CD8⁺ T cells**

CD8⁺ T cells may facilitate their anti-inflammatory function through the release of cytokines or cytotoxic mechanisms. After transfer of CD8 T cells, CD3⁺ T cells and CD68⁺ macrophages were not depleted from the tissue, excluding cytotoxicity as the underlying mechanism. We therefore explored whether cytokines released by CD8⁺ T cells mediated a suppressive effect. Anti-inflammatory cytokines described so far include IL-4, IL-10, and
TGF-β1 (18). More recently, IL-16 has been shown to provide a negative signal to CD4+ T cells (14). Of all of these cytokines, only IL-16 is primarily derived from CD8+ T lymphocytes (19, 20). CD4+ and CD8+ T cell lines were therefore analyzed for their in vitro cytokine production. Without stimulation, only mRNA of TGF-β was detectable in all lines. On polyclonal stimulation, CD4+ as well as CD8+ T cells produced IFN-γ and TGF-β but only minimal quantities of IL-4 (data not shown). IL-16 was preferentially induced in CD8+ T cells, which showed a 10-fold higher IL-16 mRNA level than did CD4+ T cells. Also, there was a trend toward higher production of IL-10 in CD8+ T cells.

To determine whether the inhibitory activity of adoptively transferred CD8+ T cells was related to the production of a cytokine, particularly IL-16, the expression of putative anti-inflammatory cytokines in synovial tissue grafts of animals injected with medium, unseparated synovial T cells, CD4+ T cells, or CD8+ T cells was compared. Results of two independent experiments are summarized in Table I. IL-10 transcripts were not detectable in any of the tissues. IL-10 transcripts were present at varying amounts without a clear correlation with the type of transferred cell. However, IL-16 and TGF-β1 transcription inversely correlated with the production of IFN-γ, IL-1β, and TNF-α mRNA. IL-16 transcripts were present in low numbers in control grafts (374 and 461 copies) but were increased 5-fold on transfer of either unseparated T cells (1910 and 2573 copies) or CD8+ T cells (1564 and 2069 copies). TGF-β1 showed a 10-fold increase in transcript numbers after the adoptive transfer of autologous synovium-derived T cells or CD8+ T cells. Transfer of CD4+ T cells did not alter the synthesis of IL-16 or TGF-β1. These data suggested that IL-16 and TGF-β1 were candidate cytokines mediating the down-regulatory activity of synovial CD8+ T cells.

We subsequently focused on IL-16, a cytokine that, so far, has not been studied in the regulation of synovial inflammation. Human synovium-SCID mouse chimeras were treated with two consecutive doses of 250 μg of anti-IL-16 Ab following the adoptive transfer of 5 × 10⁷ autologous T cells. Control mice received 250 μg of normal rabbit Ig. The results for IFN-γ from one of three independent experiments are shown in Fig. 4. Adoptive transfer of tissue-derived T cells reduced the transcription of IL-1β (data not shown) and IFN-γ to ~15% of untreated controls. In all three experiments, anti-IL-16 Ab partially reversed the T cell-mediated suppression and increased IFN-γ and IL-1β transcription. Ab treatment did not restore maximal synthesis of the proinflammatory mediators IFN-γ and IL-1β, suggesting either incomplete neutralization of tissue IL-16 or a contribution of another mediator, e.g., TGF-β1.
Expression of IL-16 in synovial CD8+ T cells

To investigate whether tissue-infiltrating T cells in rheumatoid synovitis synthesize IL-16, immunohistochemical studies were performed. Tissue sections from six patients were stained with anti-IL-16 and T cell-specific Ab. IL-16+ T cells were present in all tissues. By two-color immunohistochemistry, the phenotype of IL-16-producing T cells in the synovium was determined to be predominantly CD8+ (Fig. 5). IL-16 was found in 69–93% of all tissue-infiltrating CD8+ T cells. Only 6–19% of all IL-16-producing cells were negative for CD8. The small fraction of CD8− IL-16+ cells in the tissue included T cells as well as non-T cells, probably synoviocytes. There was a tendency for IL-16-producing CD8+ T cells to be arranged in clusters and grouped in areas of T cell enrichment. No particular spatial relationship between CD8+IL-16+ T cells and TNF-α- and IL-1β-producing CD68+ cells was detected (data not shown).

Treatment with exogenous IL-16 inhibits the production of proinflammatory cytokines in rheumatoid synovitis

If IL-16 secretion is one of the mechanisms used by CD8+ T cells to regulate the activity of the synovial immune responses, then IL-16 should be explored as a potential new treatment in RA. Synovium-SCID mouse chimeras were treated with daily injections of 500 or 1000 ng of rhIL-16; control animals received a buffer control. Therapy was continued for 10 or 14 days. For each of the cytokine doses and each of the treatment durations, four independent chimeras were studied. Following completion of the treatment protocol, the grafts were...
FIGURE 3. Frequencies of CD3$^+$ T cells, CD68$^+$ macrophages, and IFN-$\gamma$-producing and TNF-$\alpha$-producing cells in the synovial tissue after adoptive transfer of tissue-derived T cell subsets. Synovial grafts were harvested from NOD-SCID mice following the adoptive transfer of autologous tissue-derived unseparated, CD4$^+$ T cells. Serial cryosections were immunostained with anti-CD3, anti-CD68, anti-IFN-$\gamma$, and anti-TNF-$\alpha$ Ab as described in Fig. 2 legend. The number of positive cells in arbitrarily selected high power fields (hpf) was counted and is shown as mean and SD. Following the injection of autologous CD8$^+$ T cells, the frequencies of tissue-infiltrating T cells and macrophages were unchanged, but the fraction of IFN-$\gamma$- and TNF-$\alpha$-producing cells declined markedly.

Harvested, tissue cytokine mRNA was measured, and immunohistochemical studies were performed. As shown in Fig. 6, treatment with IL-16 resulted in a marked inhibition of the in situ transcription of IFN-$\gamma$, IL-1$\beta$, and TNF-$\alpha$. The inhibition was significant for all three proinflammatory cytokines for both doses and both treatment durations. Reduced transcription of all three proinflammatory cytokines was apparent after 10 days of treatment with the low dose of 500 ng of rhIL-16. The high dose of rhIL-16 (1000 ng) was slightly more effective. Notably, the inhibitory effect of IL-16 treatment on cytokine transcription was not a general phenomenon. TGF-$\beta$1 levels (Fig. 6) and IL-10 mRNA (data not shown) were unaffected by the exogenous IL-16 injection. These data also demonstrated that the increased production of TGF-$\beta$1 seen after the adoptive transfer of CD8$^+$ T cells was not a direct consequence of IL-16 release.

To evaluate the effects of exogenous IL-16 on the synovial lesions, tissue sections from synovial specimens retrieved from the treated animals were studied for the frequencies and functional activity of tissue-infiltrating T cells and CD68$^+$ macrophages. Following treatment with rhIL-16, the microanatomy of the synovial infiltrates was maintained, and the numbers of tissue-infiltrating CD3$^+$ T cells and CD68$^+$ macrophages were conserved (Fig. 7). However, injection of rhIL-16 induced a marked change in the functional activity of IFN-$\gamma$ and TNF-$\alpha$-producing cells. As shown in Fig. 7, IL-16 treatment of the chimeras resulted in an almost complete inhibition of IFN-$\gamma$ and TNF-$\alpha$ production in the inflammatory lesions.

**Discussion**

Here, we report that rheumatoid synovitis is a chronic inflammatory response that includes T cell-mediated negative feedback regulation. Making use of adoptive transfer experiments in synovium-SCID mouse chimeras, we were able to demonstrate that tissue-infiltrating CD8$^+$ T cells down-regulate the production of proinflammatory cytokines and thus attempt to function as a tissue protective mechanism. The anti-inflammatory action of CD8$^+$ T cells was partially attributed to IL-16. IL-16 is a natural ligand of the CD4 molecule that can specifically modulate the function of CD4$^+$ T cell clones, but not control clones, suggesting recognition of a specific Ag (23, 25).

Very little information is available on the involvement of synovial CD8$^+$ T cells. Also, little is known of T cell-regulatory events associated with these cells. However, the possibility that these T cells might play a role in the regulation of inflammation in RA is suggested by recent studies demonstrating that CD8$^+$ T cells are present in RA synovial tissue and are activated in the tissue and produce IFN-$\gamma$, suggesting recognition of a specific Ag (23, 25).

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**FIGURE 4.** Anti-IL-16 reverses T cell-mediated suppression of tissue cytokine transcription in rheumatoid synovitis. Tissue-derived T cells were adoptively transferred into synovium-SCID mouse chimeras as described in Fig. 1. Three days after the adoptive transfer, the mice were treated for 2 days with 250 ng of anti-IL-16 Ab or normal rabbit Ig. Tissue grafts were collected 4 days later, and cytokine mRNA was semiquantitated. Results for IFN-$\gamma$ mRNA are given as mean and SD of triplicate measurements. Data shown are representative of three independent experiments. Anti-IL-16 Abs reversed the T cell-mediated inhibition and increased IFN-$\gamma$ mRNA copy numbers.

**Table 1. Up-regulation of immune regulatory cytokines after adoptive transfer**

<table>
<thead>
<tr>
<th>Adoptive Transfer</th>
<th>Medium</th>
<th>T cells</th>
<th>CD4$^+$ T cells</th>
<th>CD8$^+$ T cells</th>
</tr>
</thead>
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<tr>
<td><strong>Expt. 1</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IL-10</td>
<td>1437 ± 131</td>
<td>1303 ± 139</td>
<td>938 ± 28</td>
<td>1764 ± 333</td>
</tr>
<tr>
<td>TGF-$\beta$1</td>
<td>597 ± 72</td>
<td>7915 ± 209</td>
<td>1045 ± 51</td>
<td>4856 ± 183</td>
</tr>
<tr>
<td>IL-16</td>
<td>461 ± 60*</td>
<td>2573 ± 544</td>
<td>694 ± 14</td>
<td>2069 ± 510</td>
</tr>
<tr>
<td><strong>Expt. 2</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IL-10</td>
<td>391 ± 14</td>
<td>395 ± 70</td>
<td>202 ± 20</td>
<td>357 ± 22</td>
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<tr>
<td>TGF-$\beta$1</td>
<td>145 ± 23</td>
<td>9840 ± 854</td>
<td>631 ± 107</td>
<td>3039 ± 81</td>
</tr>
<tr>
<td>IL-16</td>
<td>374 ± 170</td>
<td>1910 ± 71</td>
<td>427 ± 68</td>
<td>1564 ± 375</td>
</tr>
</tbody>
</table>

* Number of cytokines transcripts / 2 × 10$^6$ β-actin sequences (mean ± SD).
modifying the progression of the chronic immune response. However, recent data have implicated T cell-mediated mechanisms in disease progression as opposed to disease initiation. Careful analysis of the HLA association in RA indicated that disease progression and the clinical pattern of RA are influenced by HLA-DRB1 polymorphisms and are, consequently, under the control of T cells (26). Therefore, we were interested in regulatory events determining the outcome of synovial T cell activation. Adoptive transfer experiments using tissue-derived T cells provided the clue that T cells not only contributed proinflammatory signals but also down-regulated cytokine production. TGF-β1 and IL-16 were identified as possible mediators of this T cell-inhibitory function. Since the relevant regulatory cells were synovial CD8 and not CD4 T cells, IL-16 became a prime candidate for subsequent studies. Our results document that cytokine production in synovial lesions is greatly affected by IL-16, which, by virtue of its ability to suppress CD4-expressing cells, appears to represent a critical regulatory mechanism in rheumatoid synovitis.

The recognition that tissue-infiltrating CD8 T cells display a down-regulatory function has two important implications. First, rheumatoid synovitis may not only represent the sequela of chronic Ag recognition but may also involve a defect in feedback control mechanisms. Attempted CD8^+ T cell-mediated inhibition may explain the puzzling clinical observation that the synovial immune response takes such a slowly progressive yet destructive course. Second, the regulatory pathways involved in subduing synovial inflammation should lend themselves to be explored as novel therapeutic strategies. This concept has been proposed for monokines, e.g., IL-1 and IL-1R antagonist, as counterbalancing forces (7, 27). Whether boosting of CD8-directed immunosuppression could

FIGURE 5. Expression of IL-16 in synovial tissue CD8^+ T cells of RA patients. A, OCT-embedded cryosections of rheumatoid synovium were stained with mouse anti-CD8 mAb and rabbit anti-IL-16 Ab. Cell membrane CD8 was detected with immunoperoxidase using 3,3′-diaminobenzidine as a substrate (brown). Cytoplasmic expression of IL-16 produced a red stain when developed with alkaline phosphatase substrate kit-1. CD8^+ T cells were the major source of IL-16 in the synovial lesions. Original magnification, ×400. B, Frozen synovial tissue specimens from six RA patients were analyzed for the expression of IL-16. The mean numbers of IL-16^+ CD8^+ (solid bars) and IL-16^+ CD8^+ (open bars) cells are given. The majority of IL-16-producing cells in rheumatoid synovitis had the CD8 phenotype.

FIGURE 6. Treatment with IL-16 suppresses synovial inflammation. Synovium-SCID mouse chimeras were generated by implantation of rheumatoid synovium into NOD-SCID mice. Starting 1 wk after engraftment, the mice received daily injections of buffer control or 500 or 1000 ng of rhIL-16. Synovial tissues were retrieved after 10 and 14 days and analyzed for in situ transcription of IFN-γ, IL-1β, TNF-α, and TGF-β. Results are given as the mean of four independent experiments. Administration of IL-16 significantly inhibited the production of IFN-γ, IL-1β, and TNF-α mRNA. TGF-β transcription was unaffected by IL-16 treatment.

FIGURE 7. Effect of IL-16 treatment on the cellularity and functional activity of CD3^+ T cells and CD68^+ macrophages in synovial lesions. Synovium-SCID mouse chimeras were generated and treated with rhIL-16 as described in Fig. 6. Synovial tissue grafts were retrieved 14 days following IL-16 therapy and immunostained using the methods described in the legend to Fig. 3. The mean number of tissue-infiltrating CD3^+ T cells, CD68^+ macrophages, and IFN-γ-producing and TNF-α-producing cells were counted. IL-16 injections did not affect the survival of lesional CD3^+ T cells and CD68^+ macrophages. The numbers of IFN-γ- and TNF-α-producing cells, however, decreased markedly in grafts from IL-16-treated animals as compared with control animals.
eradicate synovial inflammation remains to be evaluated, but results from our treatment trial are encouraging.

IL-16 is a natural ligand of the CD4 molecule (11, 13). Like other CD4 ligands, such as mAb and the HIV envelope glycoprotein gp120, IL-16 can specifically regulate the function of CD4+ T cells and monocytes. Recombinant soluble CD4 and anti-CD4 mAb have been used to inhibit the biological activities of IL-16. HIV gp120 and certain anti-CD4 Abs can also mimic IL-16-mediated effects. IL-16 was originally described as a lymphocyte chemoattractant factor, and its ability to deliver positive signals was emphasized (28, 29). More recently, interest has shifted to the potential of this CD4 ligand to induce CD4+ T cell anergy (14, 30). IL-16 can suppress T cell proliferation without modulating CD4, altering IL-2 receptor expression, or affecting viability. Functional inactivation of CD4+ T cells via CD4 ligands, including IL-16, gp120, and anti-CD4 Abs, has been associated with the induction of early activation markers but with a subsequent unresponsiveness to TCR-mediated signals (30–32).

Data presented here provide an explanation for the paradoxical functional silence of synovial T cells in RA (6, 7). Provided that IL-16-mediated immunoregulation is occurring in synovitis, it can be expected that IFN-γ production is being actively suppressed. CD8+CD4+IL-16+T cells were found in synovial tissue sections from all patients. Down-regulation of IFN-γ, a CD4+ T cell product, was accompanied by inhibition of IL-1β and TNF-α, suggesting that macrophage function in rheumatoid synovitis is regulated through CD4+ T cells and that macrophage activity is a downstream effect of T cell activation. Alternatively, IL-16 may directly affect macrophages, since they also express the CD4 molecule.

IL-16 is a unique chemokine in that it is not a member of the intercrine/chemokine/IL-8 peptide family (12). It is synthesized as a precursor protein, pro-IL-16, and it is well known that CD8+ T cells secrete the bioactive form of this cytokine. T cell stimulation can activate the transcription of the IL-16 gene. Our data show that IL-16 transcription is induced after adoptive transfer of CD8+ T cells into synovium-SCID mouse chimeras, suggesting that CD8+ T cells are activated in situ, possibly by Ag-specific recognition. T cell activation is sufficient to induce the secretion of the active form of IL-16; however, additional mechanisms may be involved. Serotonin and histamine have been reported to participate in regulating the release of bioactive IL-16 (19, 20). It will be important to determine which mechanisms regulate the availability of bioactive IL-16 in the synovial microenvironment.

Results of treatment trials using rhIL-16 are promising and should encourage further exploration of this cytokine. Whether IL-16 as a monotherapy will be able to completely inhibit synovitis remains to be investigated. Treatment for only a few days was effective in suppressing cytokine production. However, short-term administration did not change the histology of the infiltrate, and prolonged treatment might be required to achieve sustained effects. If injection of IL-16 proves to be beneficial in patients with RA, a gene therapy approach of delivering this anti-inflammatory cytokine to the synovial lesions could be considered.

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