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Activation of the IL-4 STAT Pathway in Rheumatoid Synovium

Ulf Müller-Ladner,* Martin Judex,* Wibke Ballhorn,* Frank Kullmann,* Oliver Distler,* † Klaus Schlottmann,* Renate E. Gay, † Jürgen Schölmerich,* and Steffen Gay†

STATs act as second messenger after binding of a signaling molecule to its receptor. IL-4 STAT is directly involved in the IL-4-dependent gene transcription in the nucleus. We examined the expression and activation of IL-4 STAT and its related kinase Jak-1 in rheumatoid synovium. Rheumatoid arthritis (RA) synovial frozen sections of patients with short-term (<1 year) and long-term disease (>2 years) were examined using in situ hybridization and immunohistochemistry. IL-4 STAT mRNA could be detected in synovium of patients with short-term and long-term RA. The most intensive expression of IL-4 STAT mRNA could be seen in follicular inflammatory infiltrates. In the synovial lining, both fibroblasts and macrophages expressed IL-4 STAT mRNA. IL-4 STAT and Jak-1 protein was expressed by synoviocytes, and up-regulation could be induced after stimulation with IL-4. Activation of IL-4 STAT was reflected by phosphorylation of IL-4 STAT. The results indicate that IL-4 STAT is involved in key pathomechanisms in RA synovium and that IL-4 STAT-dependent pathways operate in early and late stages of the disease and presumably contribute to inhibitory immune mechanisms in RA synovium. The Journal of Immunology, 2000, 164: 3894–3901.

Rheumatoid arthritis (RA) is a progressive, destructive disease characterized by inflammation, abnormal immune responses, and synovial hyperplasia (1). Up-regulation of proinflammatory cytokines in RA synovium and synovial fluid such as IL-6 and TNF-α is a well-documented feature of active disease and intensive inflammation of the affected joints (2). However, little is known about the expression of inhibitory cytokines and their signaling pathways in synovium of RA patients. Recent results indicate that various naturally occurring cytokines and their receptors may take part in immunomodulating and inhibitory mechanisms of the immune system directed to suppress synovial inflammation and joint destruction. Among them are IL-4, IL-10, IL-1 receptor antagonist, and TNF receptor (3–5).

IL-4, which was originally named B cell growth factor (6), is a pleiotropic cytokine secreted by activated T lymphocytes, basophils, and mast cells. It regulates proliferation and specific immune functions of B cells, T cells, mast cells, macrophages, and hematopoietic progenitor cells. IL-4 activates IL-4 STAT4 by binding to its receptor and activating receptor-associated Jak kinases before tyrosine phosphorylation of IL-4 STAT, dimerization, and translocalization to the nucleus. Subsequently, IL-4 rapidly alters the pattern of gene expression by interaction with its receptor (8–11). Recently, the respective DNA binding protein has been identified and characterized (7). Examination of the amino acid sequence showed that it is a member of the STAT family of DNA binding proteins, therefore it is named IL-4 STAT. STATs, which are known to modulate various immunologic pathways (12), are located in the cytoplasm or cytoplasmic side of the cell membrane in a hypophosphorylated state, which is subject to activation by phosphorylation. Interestingly, there are indications that the effects of the IL-4/IL-4R interaction include additional tyrosine phosphorylation pathways, such as activation of Jak kinases (13, 14). Recent data also support a common STAT/Jak activation pathway, which is presumably modulated by oncogenes (15) that are also known to be up-regulated in RA synovial tissue (16).

Thus, it is feasible that IL-4 and its effector pathways might be a potential target for therapeutic intervention in RA, which prompted us to investigate the presence and expression of key molecules of the IL-4 signaling cascade (IL-4 STAT and Jak-1 kinase) in synovial tissue specimens of patients with early and late RA. In addition, to examine the functional effect of IL-4 on IL-4 STAT up-regulation, we examined IL-4 STAT phosphorylation as well as mRNA and protein synthesis in cultured rheumatoid synovial fibroblasts in an in vitro approach. Because it is known that IL-4 also affects the balance between IL-1 and IL-1 receptor antagonist (IL-1ra) in favor of IL-1ra (17), we also examined the levels of IL-1ra in culture supernatants of rheumatoid synovial fibroblasts that were stimulated with IL-4 for different amounts of time.

Materials and Methods

Tissue specimens

Tissues were obtained from 11 patients with RA who all met the revised RA criteria of the American College of Rheumatology (18) and from three patients with osteoarthritis (OA). Four of the patients had an early form of RA (disease duration, <1 year) and seven patients had long-term RA (>2 years). The tissue samples were provided by the Division of Clinical
Immunology and Rheumatology (Department of Medicine, University of Alabama, Birmingham, AL). Immediately after orthopedic arthroscopy or surgery, the tissue samples were snap frozen in OCT Tissue Tek embedding medium (Miles, Elkhart, IN) and stored at −70°C.

Preparation of riboprobes

The IL-4 STAT riboprobes were prepared by standard procedures currently used in our laboratory and described elsewhere (19, 20). In brief, cDNA fragments of IL-4 STAT (a generous gift of Dr. U. Schindler, Tularik, Inc., San Francisco, CA, (21)) were cloned into the Bluescript SK− vector (Stratagene, La Jolla, CA). After a miniprep insert check, a large-scale preparation of the IL-4 STAT containing plasmids was performed by transformation of NM 522 Escherichia coli bacteria using the calcium chloride procedure. The plasmids were extracted, purified using Nucleo- bond-AX columns (Macherey-Nagel, Düren, Germany), and linearized to permit generation of antisense and sense riboprobes.

Sense and antisense RNA probes were transcribed by T7 and T3 RNA polymerases, using a commercially available RNA transcription kit according to the manufacturer’s protocol (Stratagene). Probes were labeled with digoxigenin-UTP (Boehringer Mannheim, Indianapolis, IN). The proportion of labeled to unlabeled UTP in the reaction buffer was 1:2. The RNA was partially hydrolyzed with alkali to prepare RNA fragments of ~300 bp in length. The efficiency of the transcription was checked by gel transfer of the probe to a nitrocellulose membrane (Life Technologies, Grand Island, NY). After hybridization, the labeled RNA was detected by means of the Nucleic Acid Detection Kit (Boehringer Mannheim) according to the manufacturer’s protocol.

In situ hybridization

Frozen sections (4–6 μm) were cut and fixed in 3% buffered paraformaldehyde for 1 h at room temperature. The sections were rinsed in 2× SSC (0.3 M NaCl, 0.03 M sodium citrate) before incubation in 2× SSC (0.1 M triethanolamine-HCl (pH 8.0; Sigma, St. Louis, MO) and 0.25% acetic acid washes at room temperature, twice with 2× SSC (0.1 M triethanolamine-HCl (pH 8.0); Sigma), 2% 50% dextran sulfate (50% dextran sulfate, 50% diethylpyrocarbonate-treated double-distilled H2O (w/v); Sigma)). After the prehybridization, a rinsing step with 0.1 M triethanolamine-HCl (pH 8.0), the prehybridization was performed in prehybridization mix (50% formamide (Sigma), 20% 20× SSC (Sigma), 2% 50% Denhardt’s solution (Sigma), 5% herring sperm DNA (Sigma), stock solution 10 mg/ml, heat denatured for 10 min), 2.5% yeast tRNA (Boehringer Mannheim; stock solution 10 mg/ml), and 20% dextran sulfate (50% dextran sulfate, 50% diethylpyrocarbonate-treated double-distilled H2O (w/v); Sigma). After the prehybridization, a previously tested amount of digoxigenin-labeled IL-4 STAT heat-activated antisense probe or sense probe (for control) was applied onto the tissue sections in a 15-μl volume of prehybridization buffer. The slides were sealed with nail polish and hybridized for 2 h in a humidified chamber at 50°C. All the described steps were performed with RNase-free chemicals and in incubation chambers cleaned with 0.1% SDS (Sigma) and 100% ethanol to eliminate RNases.

After transferring the slides out of the RNase-free chambers, they were washed at room temperature, twice with 2× SSC and once with sodium-EDTA-Tris (STE)-buffer (500 mM NaCl, 1 mM EDTA, and 20 mM Tris-HCl (pH 7.5)). After digestion for 1 h at 37°C with 10% normal horse serum. The following immunogold-silver technique (Boehringer Mannheim) according to the manufacturer’s protocol was performed according to a modification of the protocol of Komminoth et al. (22). After rinsing with Tris-NaCl, the sections were incubated for 45 min with 0.1% normal horse serum to block nonspecific binding sites. All the described steps were performed with RNase-free chemicals and in incubation chambers cleaned with 0.1% SDS (Sigma) and 100% ethanol to eliminate RNases.

After rinsing with Tris-NaCl, the sections were incubated for 45 min with 0.1% normal horse serum to block nonspecific binding sites. All the described steps were performed with RNase-free chemicals and in incubation chambers cleaned with 0.1% SDS (Sigma) and 100% ethanol to eliminate RNases.

After rinsing with Tris-NaCl, the sections were incubated for 45 min with 0.1% normal horse serum to block nonspecific binding sites. All the described steps were performed with RNase-free chemicals and in incubation chambers cleaned with 0.1% SDS (Sigma) and 100% ethanol to eliminate RNases. Stainer for fibroblast markers by immunocytochemistry (18) with cathepsin G; data not shown), and tested for mycoplasms. At 70–80% confluency, cells were used for stimulation experiments as outlined below.

Cell culture

Synovial fibroblasts and tissue were obtained from synovial biopsies of patients with RA who met the criteria of the American College of Rheumatology (18). After enzymatic digestion, fibroblasts were grown in culture flasks in DMEM-Cellogro (Mediatech, Washington, DC) containing 10% FCS (Life Technologies). Cells were cultured for four passages, stained for fibroblast markers by immunocytochemistry (>95% could be stained positively for the fibroblast enzyme prolyl-4-hydroxylase, and none were positive for the macrophage marker CD68 or the neutrophil marker cathepsin G; data not shown), and tested for mycoplasmas. At 70–80% confluency, cells were used for immunocytochemistry and as outlined below.

IL-4 stimulation

Stimulation of rheumatoid synovial fibroblasts was performed in four-well chamber slides for immunocytochemistry analysis and in six-well flat-bottom culture plates for RNA analysis. Each experiment was performed in triplicate according to the following protocol. Synovial fibroblasts were grown in DMEM-Cellogro (Mediatech) for 2–3 days to achieve 70–80% confluency. Stimulation was performed using recombinant human IL-4, recombinant human IFN-γ, and recombinant human IL-4 soluble receptor (all from R&D Systems, Wiesbaden, Germany) in activity concentrations recommended by the manufacturer (IL-4, 0.2 ng/ml; IFN-γ, 1 ng/ml; soluble IL-4 receptor, 10 ng/ml; final concentrations). Each set of stimulation consisted of four assays (no stimulation, IFN-γ, IL-4, and soluble IL-4 receptor) to examine the potential of IL-4 to up-regulate intracellular IL-4 STAT expression and the in vitro inhibitory potential of the soluble IL-4 receptor. Each set of stimulation was examined at the following points: after 30 min, after 120 min, and after 360 min of exposure to the stimulatory molecules. Subsequent immunocytochemistry analysis was performed using IL-4 STAT Ab in the immunogold-silver method as described above. Controls used in immunocytochemistry were included isotypic IgG Ab and monoclonal anti-human fibroblast Ab (Diaanova, Hamburg, Germany). RNA extraction was performed using the RNeasy assay (Qiagen, Hilden, Germany) according to the protocol of the manufacturer.
RT-PCR was performed using the cDNA synthesis kit for RT-PCR (Boehringer Mannheim, Mannheim, Germany) according to the protocol of the manufacturer. RT-PCR amplification of a 496-bp segment of the IL-4 STAT gene was achieved using forward primer 5'-ACTGGAAGCAGGAAGAACTC-3' and reverse primer 5'-GGTCTGAGTCTTACCT-3' derived from published sequences (7). Examination of PCR products was performed using a 2% agarose gel and visualization by ethidium bromide, and integrity of the IL-4 STAT amplicon was examined by restriction enzyme analysis using Bst I (Stratagene) revealing two products of 235 and 262 bp, respectively.

**Immunoprecipitation and Western blotting**

In an additional set of experiments, activation of IL-4 STAT by phosphorylation after IL-4 stimulation was examined. After IL-4 stimulation as outlined above, synovial fibroblasts were lysed with ice-cold modified RIPA containing 1 mM Na-Orthovanadate (Sigma). The extracts were precleared with protein A-Sepharose beads for 1 h at room temperature. The supernatant was incubated with 8 μg anti-IL-4 STAT serum (R&D Systems) overnight at 4°C, and then Ab-protein complexes were bound to protein A-Sepharose beads, washed, and resuspended in ½ Laemmli buffer. Proteins were denatured by heating to 95°C for 10 min and separated on a 10% SDS-polyacrylamide minigel (NOVEX, San Diego, CA). Blotting was performed with 0.53 Towbin buffer containing 20% methanol in a Novex XCell II blotting apparatus for 2 h at 25 V. Thereafter, the blot was blocked in TBS containing 5% BSA for 1 h, washed with TBS/0.05% Tween, and incubated with first Ab (4G10 anti-phosphotyrosine mAb; Upstate Biotechnology, Lake Placid, NY) in TBS containing 1% BSA overnight at 4°C. After washing three times with TBS/0.05% Tween for 30 min, second Ab (goat anti-mouse HRP conjugate; Sigma) in TBS containing 1% BSA was applied. After a 1-h incubation at room temperature, the blot was washed several times in TBS/0.05% Tween, and then detection was performed with a chemiluminescence detection kit (Pierce, Rockford, IL) using an Fujifilm LAS-1000 System (Fuji-Film, Tokyo, Japan).

**FIGURE 1.** Sections of snap-frozen rheumatoid synovium specimens. A, IL-4 STAT mRNA expression; in situ hybridization technique; original magnification, ×160. A focal cluster of lymphocytes intensively expressing IL-4 STAT mRNA in an early RA synovial specimen. B, IL-4 STAT mRNA expression in an early RA synovial specimen; in situ hybridization technique; original magnification, ×300. Synovial lining cells express IL-4 STAT mRNA. The additional double labeling marks the synovial microvasculature. Arrows indicate APAAP and anti-collagen type IV Abs. C, Hematoxylin-eosin staining of a patient with long-term RA; original magnification, ×300. Strong, scattered mononuclear infiltration in the RA sublining around terminal vessels. D, Serial section of the specimen shown in C. Intensive IL-4 STAT mRNA expression in the inflamed areas (arrow). E, Snap-frozen long-term rheumatoid synovium section. Double labeling with in situ hybridization mRNA detection and APAAP immunohistochemistry; original magnification, ×640. Numerous cells, preferentially lymphocyte-shaped, that express IL-4 STAT mRNA in close proximity to small terminal vessels (double labeling performed with anti-collagen type IV Abs). F, Snap-frozen rheumatoid synovium section. Double labeling with in situ reverse transcription and APAAP immunohistochemistry; original magnification, ×800. Multiple synovial fibroblasts (arrowheads) and synovial macrophages (arrow) intensively expressing IL-4 STAT mRNA (black staining). The red staining shows the APAAP staining performed with anti-CD68 Abs. G, Serial section of the synovial sample of E and F. Hematoxylin-eosin staining illustrates the abundantly vascularized area of the synovial specimen.
performed using the NOWA chemiluminescence detection system (EnerGene, Regensburg, Germany).

The expression of IL-1ra after IL-4 stimulation was tested by ELISA (R&D Systems). Cells were grown in six-well flat-bottom culture plates and stimulated with IL-4 as outlined above. Supernatant was harvested every 30 min up to 150 min.

Results
Synovial cells from eight of the 10 patients with RA showed a positive in situ hybridization signal for IL-4 STAT mRNA. In the early RA patient group, the positively stained cells were located in focal inflammatory infiltrates (Fig. 1A) and in the lining layer (Fig. 1B). In the long-term RA patients, the most intensive accumulation of IL-4 STAT mRNA-positive cells was found within inflamed areas (Fig. 1, C and D) and, to a lesser extent, in the synovial lining (data not shown). In one patient with long-term RA, IL-4 STAT mRNA-positive cells could be detected not only in the lining layer and inflamed areas but also in the sublining. In synovium of one patient with long-term RA, IL-4 STAT mRNA-positive cells could only be detected adjacent to terminal vessels (Fig. 1, D and G).

The evaluation of whether synovial macrophages or synovial fibroblasts express IL-4 STAT mRNA by double labeling using anti-macrophage(CD68) Abs revealed that both synovial macrophages as well as synovial fibroblasts express IL-4 STAT mRNA (Fig. 1F). Protein expression of IL-4 STAT and Jak-1 (Fig. 2, A–C) could also be detected in the examined specimen in the same tissue areas (Fig. 2C), but the signal was generally less intensive than that for IL-4 STAT mRNA (Fig. 2A). Morphologically, cells expressing IL-4 STAT appeared as small mononuclear cells, fibroblasts, and macrophages (Fig. 2A). Furthermore, Jak-1 protein...
could only be seen in few, single fibroblast-shaped synovial cells (Fig. 2B).

In the synovial specimens from all three patients with OA, only a few cells expressed a positive mRNA signal for IL-4 STAT mRNA, preferentially adjacent to terminal vessels. An example is given in Fig. 3A, whereas Fig. 3B illustrates a control experiment performed with IL-4 STAT sense probe. The results for IL-4 STAT mRNA expression are summarized in Table I.

Stimulation of the cultured rheumatoid synovial fibroblasts revealed that IL-4, similar to the known effect of IFN-γ, was able to up-regulate both IL-4 STAT mRNA and protein, which could be demonstrated by RT-PCR amplification of IL-4 STAT mRNA and by immunocytochemistry. IL-4 STAT mRNA could be detected after 30 min of stimulation period, and IL-4 STAT protein was synthesized in detectable amounts after 120 min and 360 min of stimulation time. Fig. 4 shows a RT-PCR analysis of IL-4 STAT mRNA after 30 and 120 min stimulation time, and Fig. 5, A and B, shows immunocytochemical staining of IL-4 STAT protein in the nucleus of rheumatoid synovial fibroblasts after 120 and 360 min of stimulation time. Interestingly, the stimulatory effect of IL-4 resulted in a more rapid up-regulation of IL-4 STAT mRNA synthesis than stimulation with IFN-γ did (Table II and Fig. 4). Also, IL-4-induced IL-4 STAT mRNA and protein synthesis could be seen for a longer period of time than IFN-γ-induced IL-4 STAT synthesis could. After 360 min, protein expression in the IFN-γ-stimulated cultures was below the detection level. Similar to the potential of soluble IL-4R to inhibit IL-4-dependent proliferation, application of 10 ng/ml soluble IL-4R inhibited IL-4 STAT mRNA synthesis completely in the first stimulation period and led to a less intense IL-4 STAT mRNA and protein expression during the complete stimulation period. By immunocytochemistry, low IL-4 STAT protein could be detected only in a few single cells after 120 min of stimulation (Table II).

In addition, Western blotting and immunoprecipitation revealed that IL-4 STAT protein was not only expressed in considerable amounts (Fig. 6), but activation of IL-4 STAT was also confirmed by immunoprecipitation showing intensive phosphorylation of IL-4 STAT. Interestingly, phosphorylation of IL-4 STAT preceded detection of IL-4 STAT mRNA to some extent in that phosphorylated IL-4 STAT could already be detected 10 min after stimulation with IL-4 (Fig. 6), most likely reflecting two activation pathways, rapid phosphorylation of preexistent IL-4 STAT and subsequent de novo synthesis by up-regulation of IL-4 STAT mRNA. In comparison, examination of IL-1ra levels revealed no up-regulation of IL-1ra production after IL-4 stimulation of rheumatoid synovial fibroblasts.

### Discussion

Inflammation and synovial hyperplasia as well as altered humoral and cellular immune responses are hallmarks of disease activity in RA. They are associated with progressive destruction of the affected joints (1). Proinflammatory cytokines such as IL-1, IL-6, and TNF-α can be detected in significant amounts both in synovial fluid and synovial tissue in patients with RA (2, 3, 24, 25). In contrast, there are numerous indications that in RA, synovium inhibitory mechanisms are also present and activated but fail to counterregulate proinflammatory pathways in active disease (3). When augmented by therapeutic application or genetic overexpression, it could be demonstrated that naturally occurring inhibitors of cytokine effects, such as anti-cytokine Abs or soluble cytokine

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Patient No.</th>
<th>Lining Layer</th>
<th>Sublining</th>
<th>Inflammatory Infiltrates</th>
<th>Perivascular</th>
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<tbody>
<tr>
<td>Early RA (&lt;1 year; n = 4)</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>3</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td></td>
<td>4</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Long-term RA (&gt;2 years; n = 6)</td>
<td>5</td>
<td>+</td>
<td>–</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>+</td>
<td>–</td>
<td>+++</td>
<td>–</td>
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<td></td>
<td>7</td>
<td>+</td>
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<td>8</td>
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<td>9</td>
<td>–</td>
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<td>–</td>
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<td></td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>OA (n = 3)</td>
<td>11</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>–</td>
<td>–</td>
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<td></td>
<td>13</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

* +++: more than 50% positive cells; ++: 10–50% positive cells; +: 1–10% positive cells; –: no positive cells.
IL-4 was first described in 1982 as a B cell-stimulating factor (6). During the past decade, numerous reports have documented a variety of effects of this cytokine including its stimulating effect on the differentiation of T cells toward Th2 cells and the subsequent inhibition of cytokine production of Th1 cells (30–33).

In RA, IL-4 and IL-4R are present and preferentially expressed by T cells in rheumatoid synovium and synovial fluid (34, 35). Although up-regulated in mononuclear cells in blood of patients with RA (36), the dysbalance of inhibitory and proinflammatory cytokine pathways in RA synovium toward the latter is also reflected by low amounts of IL-4 (37), which has been shown to cooperate with IL-10 in prevention and reversal of cartilage degradation (5). Because synoviocytes are crucially involved in cartilage destruction (1, 38–40), this inhibitory effect might be due to blocking the synoviocyte cell cycle in early phases (41). In addition, IL-4 is capable of down-regulating IL-1, IL-6, and TNF-α production of mononuclear cells of RA patients (42–44), shedding of TNF-α p55 and then inhibition of TNF-α effects on synovial fibroblasts (45), and decrease of proinflammatory prostaglandin E2 release (46, 47). Moreover, IL-4 stimulates the development of cytotoxic lymphocytes from resting T cells (48, 49) and is involved in cytotoxic response to retrovirus-dependent tumor development (50). Because cytotoxic lymphocytes are present in RA synovium (51) and synovial fluid is known to contain hitherto unknown type C-like retroviruses (52), IL-4 effects in RA synovium may include cytotoxic activity toward viral agents.

IL-4 activates IL-4 STAT by binding to its receptor, activating receptor-associated Jak-1, before tyrosine phosphorylation of IL-4 STAT, dimerization, and translocation to the nucleus. As modulation of IL-4 and its signaling pathway is a most intriguing target for future therapies in RA, our study was performed to elucidate details of the presence, expression, and location of key molecules of the IL-4 signaling cascade. Recent characterization of the IL-4R-dependent intracellular signaling molecule IL-4 STAT facilitated the detection of mRNA for IL-4 STAT by in situ hybridization and cellular localization by immunohistochemical double labeling. The most intensive expression of IL-4 STAT mRNA could be detected in lymphocytes in inflammatory infiltrates, reflecting a highly up-regulated transcription of the IL-4 STAT gene. Interestingly, in early RA, only focal but intensive expression of IL-4 STAT mRNA could be seen in isolated inflammatory infiltrates and in perivascular lymphocytes, indicating that IL-4 STAT mRNA transcription may be one of the early events in RA. In later stages of the disease, IL-4 STAT could also be detected in synovial fibroblasts and macrophages in the lining layer and potentially could be involved in proliferation and chemotaxis of these cells (53, 54).

Tyrosine phosphorylation is an effective mechanism for activating intracellular second messengers that regulate gene transcription in response to stimuli such as cytokines. Our results demonstrate that the IL-4/IL-4 STAT pathway is active in RA synovium and that tyrosine phosphorylation is an important activation mechanism in that phosphorylated IL-4 STAT could already be detected 10 min after stimulation with IL-4 (Fig. 6), most likely reflecting two activation pathways, immediate phosphorylation of pre-existent IL-4 STAT and subsequent de novo synthesis by up-regulation of IL-4 STAT mRNA and protein. Therefore, the results support the hypothesis that the IL-4 STAT expression seen in rheumatoid synovial tissue is reflecting a continuously activated and modulated IL-4 STAT signaling pathway, presumably also involved in other inhibitory pathways known to be important for the pathophysiology of RA (55). These observations in rheumatoid synovium and synovial fibroblasts may also explain part of the situation

Table II. Expression of IL-4 STAT mRNA and protein in cultured rheumatoid synovial fibroblasts after stimulation with IFN-γ, IL-4, and a combination of IL-4 and soluble IL-4R

<table>
<thead>
<tr>
<th>IL-4 STAT mRNA</th>
<th>IL-4 STAT Protein</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>No stimulation</td>
<td>ND*</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>(+)</td>
</tr>
<tr>
<td>IL-4</td>
<td>++</td>
</tr>
<tr>
<td>IL-4 + soluble IL-4R</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, not detectable.
in synovial fluid that shows an altered balance of STAT transcription activity (56).

Interestingly, recent reports have revealed various details of the IL-4–STADependent signaling mechanisms that presumably are also effective in RA synovium. IL-4 STAD interacts with an IFN-γ activation site-like gene sequence and similarly up-regulates rapid gene transcription (57). In addition, IL-4 STAD might also be the effector pathway for insulin-like growth factor-1, which is expressed in RA synovium (58), because it is known that IL-4 and insulin-like growth factor use a common signal transduction pathway different from that of IL-4 STAD, the 4PS-pathway (59–62). This activation presumably includes the up-regulation of c-myc, a key oncogene in RA pathogenesis (63). Furthermore, our results indicate that IL-4–associated Jak kinases such as Jak-1 are also expressed in RA synovium, preferentially in synovial fibroblasts. In general, Jak tyrosine kinases act as catalytic transducers of cytokine receptor signaling (59). Because Jak kinases are also expressed in RA synovium, preferentially in synovial fibroblasts.

References


