Extravasation into Synovial Tissue Induces CCL20 mRNA Expression in Polymorphonuclear Neutrophils of Patients with Rheumatoid Arthritis

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ABSTRACT. Objective. Examination of expression of the chemokine macrophage inflammatory protein-3α (CCL20/Mip-3α) in blood polymorphonuclear neutrophils (PMN) and synovial fluid (SF) PMN of patients with rheumatoid arthritis (RA).

Methods. Paired samples of blood PMN and SF PMN were obtained from 11 patients with RA. In addition, SF was prepared from 9 patients with osteoarthritis (OA) and 10 patients with juvenile idiopathic arthritis (JIA). PMN were isolated via density centrifugation to a purity of 98%. Total RNA was isolated and the expression of CCL20 was determined by reverse transcription-polymerase chain reaction (RT-PCR) and quantitative real-time PCR. In some experiments blood PMN obtained from healthy donors were stimulated with individual SF of patients with RA. For quantitative considerations, CXCL8, CCL20, interleukin 1, and tumor necrosis factor-α (TNF-α) levels were determined in SF by ELISA.

Results. In SF of RA patients CCL20 and CXCL8 levels were elevated, up to 7.5 ng/ml and 23.6 ng/ml, respectively. No significant level of either chemokine was found in SF of patients with JIA and OA. CCL20 mRNA was undetectable in blood PMN of all patients with RA. In SF PMN, CCL20 mRNA was found in 6/11 RA patients. Expression of CCL20 mRNA in 5/6 SF PMN samples was observed in the absence of detectable TNF-α levels in SF. Cell culture experiments, however, confirmed the ability of TNF-α in SF to induce CCL20 mRNA expression in blood PMN. Notably, expression of CCL20 was also found in PMN after stimulation with SF lacking TNF-α.

Conclusion. Recruitment of PMN to the synovial microenvironment induces expression of CCL20 mRNA independent of the concentrations of TNF-α accumulating in SF of patients with RA.

Key Indexing Terms:
CCL20/MACROPHAGE INFLAMMATORY PROTEIN-3α    TUMOR NECROSIS FACTOR-α
POLYMORPHONUCLEAR NEUTROPHILS    CHEMOKINES    RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a systemic autoimmune disease, characterized by inflammation of the synovium and destruction of joint cartilage1. Although its etiology remains unre-
The inflammatory infiltrate in RA seems to persist as a consequence of recruitment, retention and enhanced survival of cells. Any of these cellular changes can be mediated by one or more chemokines, suggesting that local production of chemokines is critical for the development of rheumatoid synovitis. The relative contribution, however, of individual cell types serving as a source for chemokines in the local microenvironment of rheumatoid joints is not fully known. Some studies have identified tissue macrophages and fibroblasts as producers of different chemokines. Cell culture experiments showed that PMN can be stimulated to synthesize and secrete chemokines, including CXCL1/Gro-α, CCL2/monocyte chemotactant protein-1 (MCP-1), CCL3/macrophage inflammatory protein-1α (Mip-1α), CXCL8/IL-8, or CCL20/Mip-3α. All these chemokines have been found in SF of patients with RA, but only CXCL8 and CXCL1 have been shown to be produced by SF PMN. CCL20 is a chemotactant for dendritic cells and subsets of B and T lymphocytes, particularly CD45RO-positive memory T cells expressing CCR6.

In cell cultures, expression of CCL20 in PMN is induced by various stimuli, including TNF-α, interferon-γ (IFN-γ), formyl-methionyl-leucyl-phenylalanine (fMLP), and bacterial lipopolysaccharide (LPS). CCL20 has been detected in SF of RA patients, and expression of its receptor in synovial tissue has been implicated in the development of RA. These observations prompted us to investigate the expression of CCL20 in PMN of RA patients with regard to fluid-phase TNF-α. The results show expression of CCL20 in SF PMN, but not in blood PMN of the same patients. There was, however, no correlation between TNF-α levels in SF and CCL20 mRNA expression in SF PMN, suggesting that local TNF-α does not contribute to CCL20 expression in SF PMN.

**MATERIALS AND METHODS**

**Patients.** Blood and SF samples were obtained from 11 RA patients with active disease; all patients gave informed consent. The severity of symptoms was classified according to the Disease Activity Score 28 (DAS28). All available DAS28 scores were >5.3 and revealed a high disease activity. Patients were divided into 2 groups with respect to therapy (Table 1). Six patients were receiving anti-TNF therapy and 5 patients were treated with conventional therapy (nonsteroidal antiinflammatory drugs, steroids, disease-modifying antirheumatic drugs). Qualitative estimates of cell types accumulating in SF were assessed by light microscopy after Diff-Quick staining (Dade Behring, Dübeningen, Switzerland). For quantitative considerations, total numbers of PMN in SF were determined after Percoll fractionation (Amersham Biosciences, Freiburg, Germany). SF of 9 patients with osteoarthritis (OA) and 10 patients with juvenile idiopathic arthritis (JIA) served as control.

**Purification of PMN from blood and SF.** Purification of PMN from blood was done as described with minor modifications. For sedimentation of erythrocytes, 1 vol EDTA blood was incubated with 1 vol 3% Dextran T500 (Roth, Karlsruhe, Germany) in phosphate buffered saline (PBS; Biochrom, Berlin, Germany) for 20 min at 4°C. The leukocyte-rich supernatant was centrifuged at 500 × g for 10 min at 6°C in a Multiuge (Heraeus, Hanau, Germany). The pellet was dissolved in PBS, overlaid onto an isotonic discontinuous Percoll gradient with densities of 1.075 g/ml and 1.09 g/ml and centrifuged at 750 × g for 25 min at 6°C. PMN were collected at the lower interphase and washed twice with PBS.

With the exception of dextran sedimentation, preparation of SF PMN was done using the same procedure as described for blood PMN. Cell-free SF was obtained by centrifugation of SF at 750 × g for 25 min at 4°C and stored at −70°C in aliquots until use.

**FACS analysis.** Purity of PMN was routinely analyzed by flow cytometry with FACSCalibur (BD Biosciences, Heidelberg, Germany). Briefly, to prevent nonspecific binding, 3 × 10^6 cells were preincubated with heat inactivated (20 min, 56°C) human serum for 10 min and subsequent incubation with a combination of FITC conjugated CD66b antibody (Immunotech, Hamburg, Germany) and APC conjugated CD14 antibody (Caltag, Hamburg, Germany). After 30 min at 4°C, cells were washed twice with PBS/1% fetal calf serum (Sigma, Deisenhofen, Germany) and kept on ice until analysis. Data were analyzed with CellQuest software (BD Biosciences) revealing a purity of 98%–99% CD66b-positive PMN with a contamination of < 0.05% CD14-positive and CD66b-negative cells. In some experiments, a similar degree of purity was obtained when PMN were prepared via Percoll fractionation in combination with CD16 MACS antibody (Miltenyi Biotec, Bergisch Gladbach, Germany) cell sorting. Additionally, preparations of SF PMN of most patients were subjected to histochemistry (Diff-Quick staining) to eventually detect other contaminating cell types, including eosinophils, monocytes, and lymphocytes. We did not observe significant numbers of these cell types in PMN preparations.

**Cell culture conditions.** For culturing PMN, RPMI 1640 (Biochrom) was supplemented with 5 mM Hepes (Sigma), 2 mM L-glutamine (Biochrom), 100 U/ml penicillin, 100 µg/ml streptomycin (Biochrom), and 0.2% NaHCO_3 (Sigma). Blood PMN from healthy blood donors were seeded in 24 well plates (Corning Costar, Bodenheim, Germany) in 500 µl culture medium with or without 10 ng/ml TNF-α, or in 500 µl SF of RA patients with or without 50 µg/ml anti-TNF-α antibody (inflimiximab). In validation studies this antibody concentration was able to block CCL20 expression induced by 10 ng/ml recombinant TNF-α in blood PMN. For preparation of mRNA, PMN were seeded at a density of 8 × 10^6 cells. Additionally, to analyze cell associated CCL20, PMN were cultured at a density of 1 × 10^7 cells when stimulated with TNF-α and at a density of 3 × 10^6 when stimulated with SF. After 4 h or 42 h of incubation (37°C, 5% CO_2, 95% humidity) cells were removed for subsequent RNA isolation or ELISA.

**ELISA analysis.** Cell lysates were generated with 100 µl PBS containing 10 mM EDTA (Merk, Darmstadt, Germany), 0.2% Triton (Sigma), and proteinase inhibitors (Roche, Mannheim, Germany) and 3 subsequent cycles of freezing and thawing. CXCL8 and CCL20 levels in cell lysates and SF were quantified using human IL-8 ELISA (OptEIA™ Set Human IL-8, BD Biosciences) and ELISA development kit for CCL20 (DuoSet®-R&D Systems, Wiesbaden-Nordenstadt, Germany), respectively, according to the manufacturer’s instructions. Substrate reagents A and B were from BD Biosciences. Each assay sensitivity was about 5 pg/ml. For measurement of TNF-α and IL-1, SF were subjected to sandwich ELISA using capture antibodies and biotinylated detection antibodies from Endogen (Bonn, Germany). TNF-α and IL-1β (standard) were from R&D Systems. Peroxidase conjugated streptavidin-biotin complex was obtained from Dako (Hamburg, Germany) and N-300 substrate solution was from Endogen. The sensitivity of the TNF-α and IL-1β ELISA was 15 and 30 pg/ml, respectively.

**Reverse transcription polymerase chain reaction (RT-PCR).** Total RNA from PMN was extracted with RNeasy mini-spin columns (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. In some experiments RNA was prepared using the acid-phenol extraction procedure. All RNA samples were digested with RNase (RNA-free™ kit; Ambion, Huntingdon, UK). RNA yields were determined spectrophotometrically by measuring the absorbance at 260 and 280 nm. The cDNA was synthesized from 0.25 µg of total RNA using 0.5 µg oligo (dT) 16-mer primer (Thermo, Ulm, Germany), 0.5 mM dNTP (Invitrogen, Karlsruhe, Germany) and 1 U Omniscript reverse transcriptase (Qiagen) in a final volume of 25 µl at 37°C for 50 min. Reverse transcription was terminated by heating the reaction mix at 95°C for 5 min.
3°C for 60 min and 93°C for 5 min. PCR was performed in a 25 µl reaction mixture containing 2 µl cDNA samples, 0.625 U Taq polymerase (Qiagen), 0.2 mM dNTP (Invitrogen), and 0.4 µM each of the sense and antisense primers. Amplification was carried out at 95°C for 2 min followed by 35 cycles of denaturing at 94°C for 1 min, annealing at 60°C for 50 s, and extension at 72°C for 90 s. A final extension step was performed at 72°C for 7 min on a thermocycler (Biozym; Hess, Oldendorf, Germany). PCR samples were separated on 2% agarose gels (Roth), visualized by ethidium bromide staining (Merck), and photographed on an ImageMaster VDS (Amersham Biosciences).

Quantitative real-time RT-PCR was performed in a 20 µl reaction mixture containing 1 µl cDNA, 1 U Platinum-Taq-polymerase (Invitrogen), 0.5× SYBR-Green (Roche), 5% DMSO (Sigma), 0.5 mg/ml bovine serum albumin (New England Biolabs, Frankfurt, Germany), 0.25 mM dNTP (Amersham), 4 mM MgCl₂, and 0.4 µM each of the sense and antisense primers. Amplification was carried out on a real-time light cycler (Roche) at 95°C, 10 min, 20°C/s of preincubation followed by 50 cycles of denaturing at 95°C, 15 s, 20°C/s, annealing at 53°C (β-microglobulin) and 60°C (CCL20), 10 s, 20°C/s, and extension at 72°C, 15 s, 20°C/s. The melting curve was performed at 95°C, 0 s, 20°C/s followed by 65°C, 15 s, 20°C/s and 95°C, 0 s, 0.1°C/s. Cooling was done at 40°C, 30 s, 20°C/s. CCL20 values were normalized with β-microglobulin values and denoted as times-fold difference in mRNA expression between the samples.

RESULTS

CCL20 levels are elevated in SF of RA patients. Initial experiments were performed to assess the levels of CCL20 in SF of patients with different forms of arthritis. As shown in Figure 1A, CCL20 concentrations were elevated in RA patients, with mean values of 7.5 ng/ml for the anti-TNF treatment group and 5.4 ng/ml for those patients under conventional therapy. No or little CCL20 was detectable in SF of patients with OA (n = 9) or JIA (n = 10). In comparison, high levels (mean 23.6 ng/ml) of CXCL8 were found only in SF of RA patients under conventional therapy, while significantly lower levels of CXCL8 were detectable in SF of RA patients with anti-TNF therapy (mean 2.59 ng/ml). No or little CXCL8 was measured in SF of patients with OA or JIA (Figure 1B).

Induction of CCL20 expression in SF PMN of RA patients. The expression patterns of CCL20 were investigated in SF PMN by RT-PCR of total RNA in comparison to expression of CCL2 and CXCL8. As shown in Figure 2A, SF PMN expressed CXCL8 and CCL2 mRNA in 10/11 and 9/11 patients, respectively. CCL20 mRNA expression was found in SF PMN of 6 patients to a variable extent (only faint bands in Patients 6 and 10). Quantitative real-time RT-PCR

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Patient</th>
<th>Sex, Age</th>
<th>Medication</th>
<th>SF Cell Type, % (PMN/Ly/Mo+Ma)</th>
<th>SF PMN Count × 10⁶/ml SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-TNF</td>
<td>1 F, 44</td>
<td>NSAID: ibuprofen</td>
<td>38/24/38</td>
<td>3.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 F, 56</td>
<td>Steroids: cortisone</td>
<td>60/22/18</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 F, 66</td>
<td>Anti-TNF-α: infliximab</td>
<td>90/8/2</td>
<td>5.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 F, 32</td>
<td>Anti-TNF-α: adalimumab</td>
<td>84/5/11</td>
<td>2.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 F, 40</td>
<td>Anti-TNF-α: infliximab</td>
<td>ND</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 F, 49</td>
<td>Anti-TNF-α: adalimumab</td>
<td>87/9/4</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>2 F, 67</td>
<td>NSAID: indomethacin</td>
<td>76/17/7</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 F, 71</td>
<td>Steroids: cortisone</td>
<td>83/11/6</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 F, 71</td>
<td>DMARD: sulfasalazine</td>
<td>80/10/10</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 M, 57</td>
<td>DMARD: azathioprine</td>
<td>72/18/10</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 M, 51</td>
<td>DMARD: methotrexate</td>
<td>95/2/3</td>
<td>25.2</td>
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</tr>
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</table>


Table 1. Clinical data of 11 patients with RA providing blood and SF samples. SF analysis was performed by light microscopy after DiffQuick staining. Cell differentiation is described as a percentage of PMN, lymphocytes (Ly), and monocytes/macrophages (Mo+Ma). SF PMN counts are after Percoll fractionation.
was performed for Patient 7 with strong and Patient 10 with weak CCL20 mRNA expression. Results revealed a 10,000-fold difference of CCL20 mRNA expression in SF PMN between Patients 7 and 10. When compared to blood PMN of the same individuals (Figure 2B), no CCL20 mRNA was detectable, indicating that CCL20 mRNA expression is upregulated after recruitment into the inflamed joint. CXCL8 mRNA seemed to be constitutively expressed in blood PMN and SF PMN as well. In this context, CXCL8 mRNA, but not CCL2, was frequently found even in healthy donors (data not shown).

In addition, no correlation was found between IL-1ß and CCL20 levels in SF or CCL20 mRNA expression in SF PMN (Table 2).

Finally, because induction of CCL20 mRNA was primarily found in SF PMN of patients under conventional therapy, we speculated that TNF-α contributes to the induction of CCL20 expression in SF PMN. To address this issue, TNF-α levels were measured in SF of each patient. The results (Table 2) show detectable levels of TNF-α primarily in SF of patients of the anti-TNF therapy group, and in one patient of the conventional therapy group. However, none of these patients strongly expressed CCL20 mRNA in SF PMN. By contrast, high expression of CCL20 mRNA was found in PMN prepared from SF with undetectable levels of TNF-α (Patients 7, 8, 9, and 11).

To analyze cell associated CCL20, cell lysates of SF PMN from 3 RA patients were subjected to ELISA. The results depicted in Figure 3A revealed that only the lysate of SF PMN of Patient 5 contained substantial amounts of cell associated CCL20. The measured 50 pg/ml in this lysate of SF PMN was about half the amount of cell associated CCL20 measured in lysates of blood PMN after stimulation with TNF-α (Figure 3B). This indicates that PMN of RA patients have the capacity to produce CCL20 in SF of RA patients. SF-induced CCL20 expression in blood PMN of healthy donors. CCL20 mRNA expression was determined in blood PMN of healthy donors after incubation with SF containing

![Figure 1. Levels of CCL20 (A) and CXCL8 (B) in SF of RA patients with anti-TNF therapy (n = 6), RA patients with conventional therapy (n = 5), OA patients (n = 9), and JIA patients (n = 10) was determined by ELISA. Chemokine levels are shown as mean ± SEM and analyzed by unpaired t test (2 tailed p value). Significant differences *p < 0.05 and **p < 0.005.](https://www.jrheum.org)
Figure 2. Expression of CCL2, CCL20, and CXCL8 mRNA by PMN of patients with RA. Total RNA was prepared from SF PMN (A) and blood PMN (B) of 6 patients undergoing anti-TNF therapy (left panels) and 5 patients with conventional therapy (right panels) and subjected to RT-PCR with primers for CCL2, CCL20, CXCL8, and actin. No blood sample was available from Patient 10. PCR was repeated at least 3 times.

Table 2. TNF-α, CXCL8, CCL20, and IL-1ß levels in SF of patients with RA with anti-TNF therapy (n = 6) and conventional therapy (n = 5) were determined by ELISA. CCL20 mRNA expression in SF PMN is denoted according to Figure 2A.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>TNF-α in SF</th>
<th>CXCL8 in SF</th>
<th>CCL20 in SF</th>
<th>IL-1ß in SF</th>
<th>CCL20 mRNA in SF PMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-TNF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>Ø</td>
<td>2.57</td>
<td>1.588</td>
<td>68.33</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>Ø</td>
<td>0.73</td>
<td>3.269</td>
<td>86.66</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>395.5</td>
<td>6.16</td>
<td>7.987</td>
<td>88.33</td>
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</tr>
<tr>
<td>6</td>
<td>813.6</td>
<td>1.44</td>
<td>1.856</td>
<td>71.66</td>
<td>+/-</td>
</tr>
<tr>
<td>10</td>
<td>Ø</td>
<td>1.18</td>
<td>8.99</td>
<td>76.66</td>
<td>+/-</td>
</tr>
<tr>
<td>11</td>
<td>Ø</td>
<td>3.51</td>
<td>24.837</td>
<td>71.66</td>
<td>+</td>
</tr>
<tr>
<td>Conventional</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 2</td>
<td>Ø</td>
<td>6.6</td>
<td>2.876</td>
<td>88.33</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>66.4</td>
<td>31.9</td>
<td>3.379</td>
<td>Ø</td>
<td>–</td>
</tr>
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<td>7</td>
<td>Ø</td>
<td>37.11</td>
<td>10.212</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Ø</td>
<td>40.62</td>
<td>8.871</td>
<td>70</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Ø</td>
<td>1.59</td>
<td>1.556</td>
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<td>+</td>
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</tbody>
</table>

Ø: No TNF-α or IL-1 detected. –: No CCL20 mRNA detected. +/-: Low CCL20 mRNA detected. +: CCL20 mRNA detected.
either high (Patient 5) or undetectable levels of TNF-α (Patients 7 and 8). The results show induction of CCL20 mRNA with SF containing TNF-α, which was completely blocked in the presence of anti-TNF antibodies (Figure 4A). Remarkably, SF PMN of this patient showed no CCL20 mRNA (Figure 2A). In comparison, SF lacking TNF-α from Patients 7 (Figure 4b) and 8 (data not shown) were unable to induce CCL20 mRNA in blood PMN. Interestingly, though, the levels of CCL20 in cell lysates were different when stimulated with either SF 5 or SF 7. Anti-TNF did not affect the protein levels after stimulation with SF 5 and SF 7 as well (Figure 4C). The results confirm TNF-α as an inducer for CCL20, but further argue for a TNF-α-independent CCL20 expression.

DISCUSSION

This study identifies SF PMN as a possible source for CCL20 in patients with RA. In circulating PMN, expression of CCL20 mRNA was undetectable, indicating that recruitment of PMN into the local inflammatory environment is a prerequisite for the induction of CCL20 expression. In contrast, CCL2 transcripts were readily detectable in blood PMN of most patients, supporting the notion of a disease-associated priming of circulating PMN in RA. The data extend earlier findings showing that CCL20 can be produced in synovial tissue and infiltrating mononuclear cells of patients with RA. In addition, consistent with previous observations, we observed that elevated concentrations of CCL20 accumulate in SF of patients with RA, while no significant amounts of CCL20 could be measured in SF of patients with either OA or JIA. The data suggest that CCL20 plays a role in the pathogenesis of RA, but not in other types of arthritis. As RA and JIA seem to be more closely related to each other than OA and JIA in terms of pathogenesis, this result was unexpected. Patients with RA and JIA in this study apparently differ according to their age as well as in their treatment. No patient with JIA received steroids, disease modifying antirheumatic drugs, or anti-TNF treatment at the time of joint fluid sampling. The expression of CCR6, the ligand for CCL20, was shown to be age-dependent.

From studies in animal models there is general agreement that TNF-α is crucial in RA pathogenesis. Blocking the effects of TNF-α leads to a reduction of the inflammatory response concomitant with decrease of joint inflammation and destruction. Finally, convincing evidence comes from studies showing clinical benefit for RA patients treated with the anti-TNF monoclonal antibodies. The effects mediated by TNF-α are part of an array of different responses, including induction of CXCL8, the principal mediator of neutrophil migration. From measurements of CXCL8 levels in SF of RA patients we provide evidence that CXCL8 accumulates in SF of patients undergoing conventional therapy, but not in SF of patients with anti-TNF therapy. The data are consistent with previous observations that TNF-α blockade reduces synovial tissue expression of the chemokine CXCL8. Even so, remarkably high levels of TNF-α were found in SF of some patients undergoing anti-TNF therapy, but not in patients with conventional therapy. These findings are consistent with recent studies indicating that circulating TNF-α levels are elevated in RA patients after injection of a single dose of anti-TNF monoclonal antibody. In addition, there is evidence that elevated TNF-α levels in patients with anti-TNF therapy correlate with reduced disease activity. Consistent with these observations, our studies show that patients with elevated TNF-α levels in SF (Patients 5 and 6) have little CXCL8 (Table 2).

In cell culture experiments, Scapini, et al have shown that TNF-α can serve as a potent mediator of CCL20 mRNA expression in blood PMN. CCL20 transcripts, however, were highly unstable, unless PMN were costimulated with LPS and either IFN-γ or fMLP. It is thus possible that detection of CCL20 transcript in the conventional therapy group relates to the presence of at least one factor, such as IFN-γ, with a stabilizing activity for CCL20 mRNA.
Our cell culture experiments also showed that incubation of blood PMN with SF from Patient 5 induced CCL20 mRNA expression. The stimulatory activity could be attributed to TNF-α, because CCL20 mRNA expression in PMN was completely blocked by anti-TNF antibodies. For SF from other patients, e.g., from Patient 7, no TNF transcripts were detectable, although CCL20 protein was found in cell lysates after prolonged incubation, indicating that SF from Patient 7 apparently lacked CCL20 mRNA-stabilizing activity.

Analysis of cell associated CCL20 revealed that SF PMN of one RA patient contained a substantial amount of CCL20, confirming that SF PMN can serve as a source of CCL20 in SF. In lysates of SF PMN of 2 other RA patients, CCL20 levels ranged from 15 to 25 pg/ml and were considered background, as judged from CCL20 levels measured in lysates of unstimulated blood PMN of healthy donors. Differences in the levels of cell associated CCL20 between individual RA patients may result from either compromised cell viability or differentiation of recruited PMN into a different phenotype.

There was no significant difference between CCL20 levels in SF of RA patients with anti-TNF treatment and those with conventional therapy. Additionally, although CCL20 mRNA was predominantly expressed in SF PMN of patients with conventional therapy, no SF TNF-α was found in these patients. Therefore, it is tempting to speculate that in the synovial environment TNF-α does not account for induction of CCL20 in SF PMN. Several explanations include: (1) TNF-α in SF may be biologically inactive as a consequence of anti-TNF therapy. This seems most unlikely, however, as TNF-α in SF was able to induce CCL20 mRNA in blood PMN in vitro. (2) SF PMN develop into a different phenotype that, for instance, contains high levels of MHCII mRNA and intracellular MHCII protein, and therefore may be resistant to TNF-α due to an inactive TNF receptor. (3) It is also possible that the interaction with surface molecules of epithelial cells or fibroblasts changes the ability of SF PMN to express CCL20. This is consistent with the finding that SF PMN cultured with SF fibroblast-like synoviocytes showed increased expression of Mip-1α via a β2-integrin/intercellular adhesion molecule 1 mediated mechanism. (4) Moreover, stimulation of blood PMN with SF lacking TNF-α was also able to induce CCL20 expression, suggesting that other mediators (such as IFN-γ) may contribute to CCL20 expression.

Data from this study identify SF PMN as a possible cellular source of CCL20 in inflamed joints of patients with RA. Induction of CCL20 mRNA expression arises as part of the recruitment process of PMN into the synovium. CCL20 mRNA expression in SF PMN occurs in the absence of TNF-α, which may raise the question whether CCL20 expression in PMN is not sensitive to anti-TNF treatment of patients with RA.

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REFERENCES