Fatal attraction: chemokines and rheumatoid arthritis
Haringman, J.J.

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Chapter 5

EFFECTS OF ORAL PREDNISOLONE ON BIOMARKERS IN SYNOVIAL TISSUE AND CLINICAL IMPROVEMENT IN RHEUMATOID ARTHRITIS

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ABSTRACT

Objective To create greater understanding of the changes in synovial tissue parameters that occur in conjunction with clinical response by using an effective therapy, in order to facilitate the planning of future studies with therapeutic agents for rheumatoid arthritis (RA).

Methods Twenty-one patients with active RA were randomized to receive either oral prednisolone (n = 10) or placebo (n = 11) for 2 weeks. In all patients, synovial tissue biopsy specimens were obtained by arthroscopy directly before treatment and after 14 days of treatment. Immunohistochemical analysis was performed to characterize the cell infiltrate and vascularity. Stained tissue sections were analyzed by digital imaging. Statistical analysis was performed using an analysis of covariance model.

Results After treatment, the mean Disease Activity Score in 28 joints (DAS28) was 2.0 units lower (95% confidence interval [95% CI] 1.0-3.0) in patients who received prednisolone than in those who received placebo. In the prednisolone group, the mean (±SD) DAS28 decreased from 6.27 ± 0.95 to 4.11 ± 1.43 after therapy; minimal change was observed in the placebo group. For macrophages, the estimated effect of prednisolone was large. Patients receiving active treatment had fewer (mean 628 cells/mm² [95% CI 328-927]) macrophages after therapy compared with those receiving placebo. A reduction in the total number of CD68+ macrophages, from 1.038 ± 283 cells/mm² before treatment to 533 ± 248 cells/mm² after treatment, was observed in the prednisolone group. There were clear trends toward decreased infiltration by T cells, plasma cells, and fibroblast-like synoviocytes after active treatment. We observed a trend toward a reduction in αvβ3+ newly formed blood vessels and expression of vascular growth factors after prednisolone therapy.

Conclusion Prednisolone therapy in RA is associated with a marked reduction in macrophage infiltration in synovial tissue, suggesting that synovial macrophage numbers could be used as a biomarker for clinical efficacy.

INTRODUCTION

In recent years, there has been increased interest in studying the synovial tissue of patients with rheumatoid arthritis (RA). Reasons for this include the recognition of the synovium as the primary site of inflammation and technical developments that made it possible to study the processes that take place in the synovial tissue in more detail. Although examination of peripheral blood and synovial fluid may also provide insight into the production of soluble mediators as well as the dynamics of migration of inflammatory cells into different compartments, it has become clear that such studies yield only indirect information about the events in the synovium (1). In addition to the use of synovial biopsies for diagnostic purposes (2;3) and pathogenetic studies (4), serial synovial biopsy has been used to evaluate the effects of novel treatments (5).

The serial synovial biopsy approach has been proposed as a method that might help determine potential effects of novel antirheumatic interventions. The increase in the development of a variety of new, targeted therapies clearly raises the need for sensitive biomarkers, which could be used for selection purposes during the development process. In the past, successful treatment of RA with disease-modifying antirheumatic drugs (DMARDs) and biologics was associated with a decrease in mononuclear cell infiltration of the synovial tissue. Of interest, treatment with high-dose intravenous methylprednisolone was found to reduce the expression of tumor necrosis factor-alpha (TNF-α) in the intimal lining layer and synovial sublining as soon as 24 hours post treatment, with associated clinical improvement (6).
It is important to note that analysis of serial synovial samples obtained from RA patients who received either placebo or in whom treatment with low-dose anakinra or interleukin-10 (IL-10) was unsuccessful did not reveal any changes in the synovial tissue (7,8). The observation that synovial inflammation can occasionally be reduced to some extent in the absence of clinical improvement (9), or with only a modest decrease in serum levels of acute-phase reactants (10), suggests that examination of serial synovial biopsy specimens might be more sensitive to change compared with clinical response criteria. These studies suggest that analysis of serial biopsy specimens could be used as a screening method to test new compounds, requiring relatively small numbers of subjects. The absence of changes in the synovial tissue after treatment would suggest that the therapy is probably not effective.

The objective of this study was to provide a greater understanding of the changes that occur in the synovial tissue in association with clinical response by using a known clinically effective therapy, to find sensitive biomarkers that may facilitate the planning of future studies with novel agents to treat RA. In addition, this study is the first to investigate the effects of treatment with oral corticosteroids, according to the Combinatietherapie Bij Reumatoïde Artritis (COBRA) schedule (11), on rheumatoid synovium.

PATIENTS AND METHODS

Patients.
Patients with RA diagnosed according to the 1987 criteria of the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) (12), ages 18-85 years, were included in the study if they had Steinbrocker functional class I-III RA (13). All patients had active disease at the time of enrollment, defined by the presence of ≥ 6 tender joints and ≥ 6 swollen joints of 28 joints assessed. Additional inclusion requirements were an erythrocyte sedimentation rate (ESR) of at least 28 mm/hour or serum levels of C-reactive protein (CRP) of at least 1.5 mg/dl, or morning stiffness that lasted for at least 45 minutes. Patients had to have received a stable dosage of DMARDs such as methotrexate, sulfasalazine, hydroxychloroquine, leflunomide, or a combination of these for at least 28 days. Use of nonsteroidal anti-inflammatory drugs was allowed, provided that the dose had been stable for at least 28 days.

Parenteral, intra-articular, or oral use of corticosteroids within 28 days before enrollment in the study was not allowed. Other exclusion criteria were acute major trauma within 28 days before entering the study, the use of any investigational drug (such as monoclonal antibodies [e.g., TNF inhibitors], growth factors, or cytokines) within the previous 3 months, and a history of glaucoma, psychosis, mania or severe depression, epilepsy, cerebrovascular disease, tuberculosis, or cancer. Patients with uncontrolled high blood pressure (defined as systolic pressure of >160 mm Hg and diastolic pressure of >95 mm Hg), insulin-dependent or poorly controlled non-insulin-dependent diabetes, a history of confirmed peptic ulceration, or known adverse reactions to prednisolone or drugs with a chemical structure similar to that of prednisolone were excluded from the study. Additional exclusion criteria were clinically significant abnormalities in laboratory assessments, hematologic disease, immunodeficiency, or impaired coagulation.

Study protocol.
This double-blind, placebo-controlled, single-center study was performed in the Academic Medical Center of the University of Amsterdam. The study protocol was approved by the
Medical Ethics Committee of the institute, and all patients provided informed consent before the start of the study. For each patient, a complete medical history was obtained and a full physical examination, including joint assessment, was performed. Clinical chemistry, hematology, and coagulation assessments, urinalysis, chest radiography, and skin testing for tuberculosis were performed. Eligible patients were included in the study within 1 week and were randomized to receive either a course of oral prednisolone, 60 mg daily for the first week followed by 40 mg daily during the second week (similar to the dosages used in the COBRA study (11)), or matching placebo for 2 weeks.

Clinical assessment included a 28-joint count for tenderness and swelling, the patient's score for pain on a 100-mm visual analog scale (VAS; 0 = no pain, 100 = worst possible pain), patient's and physician's scores for global assessment of disease activity on a 100-mm VAS (0 = very well/no arthritis activity, 100 = very poor/extremely active arthritis), and a 4-point Health Assessment Questionnaire (14) for quality of life (0 = no difficulty, 3 = severe disability). Furthermore, CRP levels and ESRs were determined. An independent assessor performed the clinical evaluation.

Treatment.
Patients were randomized in a 1:1 ratio to receive either a 2-week course of oral prednisolone (60 mg/day for the first week followed by 40 mg/day during the second week) or matching placebo.

Blood cytokines, matrix metalloproteinase 3 (MMP-3), and urinary markers of bone degradation.
Cytokine levels (IL-1β, IL-1α, IL-6, IL-8, IL-18, and TNF-α) were measured in serum by the Luminex (Luminex, Austin, TX) or enzyme-linked immunosorbent (ELISA) method, and levels of MMP-3 were measured in serum by ELISA. Changes in urinary markers of bone degradation (pyridinoline, deoxypyridinoline, the creatinine-to-pyridinoline ratio, and the creatinine-to-deoxypyridinoline ratio) were assessed. Creatinine was measured by the Jaffe method, a kinetic reaction. Pyridinoline and deoxypyridinoline levels were measured by enzyme immunoassays.

Arthroscopy.
In all patients, needle arthroscopy of an actively inflamed joint (knee, ankle, or wrist) was performed with the patient under local anesthesia, before treatment and after 14 days of treatment (in the same joint). The procedures for needle arthroscopy and tissue processing were performed as described previously in detail (15;16). During each procedure, biopsy specimens were obtained from ≥ 6 sites in the joint to minimize sampling error (17;18).

Immunohistochemical analysis.
In order to analyze the cell infiltrate, serial sections were stained with the following antibodies: anti-CD68 (EMB11; Dako, Glostrup, Denmark), anti-CD163 (Ber-MAC3; Dako), anti-CD14 (clone M5E2; Becton Dickinson, Erembodegem, Belgium), anti-CD64 (clone 10-1; Dako), anti-CD32 (KB61; Dako), anti-CD5 (LI7F12; Becton Dickinson), anti-CD4 (SK7; Dako), anti-CD8 (DK25; Dako), anti-CD22 (CLB-B-ly/1.6B11; Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), anti-CD38 (HB-7; Becton Dickinson), anti-CD138 (B-B4; Immunotech, Marseilles, France), anti-CD3 (SK7; Becton Dickinson), anti-CD55 (clone 67; Immunotech), and anti-granzyme B (GrB-7; Monosan, Uden, The Netherlands).
For immunohistochemical analysis of cytokine expression, IL-1β (anti-IL-1β, 2D8; Immunokontact, Stockholm, Sweden), IL-1α (anti-IL-1α, 1277-82-29; Immunokontact), and
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TNF-α (anti-TNF-α, 52B83; Monosan) were stained. Furthermore, several markers of angiogenesis (19) were analyzed: αvβ3 (23C6; Santa Cruz Biotechnology, Santa Cruz, CA), von Willebrand’s factor (F8/86; Dako), basic fibroblast growth factor (bFGF; clone 6; Transduction Laboratories, Lexington, KY), and vascular endothelial growth factor (VEGF; C-1; Santa Cruz Biotechnology). Additionally, the expression of chemokine receptor CCR1 (53504.11; R&D Systems, Abingdon, UK), MMP-1 (41-1E5; Oncogene Research Products, Cambridge, MA), and MMP-13 was determined.

Sections with non-assessable tissue, defined by the absence of an intimal lining layer, were not analyzed. For control sections, the primary antibodies were omitted, or irrelevant antibodies were applied. Staining for cellular markers was performed using a 3-step immunoperoxidase method as previously described (9). For the determination of cytokine expression, biotinylated tyramine was used for amplification, as previously described (20:21).

Digital image analysis.

After immunohistochemical staining, all coded sections were randomly analyzed by computer-assisted image analysis. For all markers, 18 high-power fields were analyzed. CD68 expression was analyzed separately in the intimal lining layer and the synovial sublining. The images of the high-power fields were analyzed using the QWin image analysis system (Leica, Cambridge, UK), as described previously in detail (22:23).

Statistical analysis.

Each of the end points was statistically analyzed using an analysis of covariance (ANCOVA) model. The model included terms for treatment as a fixed effect and the baseline measurement as a covariate. The aim was to assess the treatment difference.

RESULTS

Demographic features.

Twenty-one patients (8 men, 13 women) were included in the trial within 18.4 ± 21.9 days. Ten patients received prednisolone, and 11 patients received placebo. The mean ages were 49.4 years (range 32-63 years) for the patients in the prednisolone group and 55.7 years (range 37-69 years) for those in the placebo group (Table 1). The median durations of disease and DMARD use were comparable in both groups. None of the patients had received TNF inhibitors in the past. All patients had active disease, with similar levels of disease activity in both groups (Table 1). The ESRs and CRP values were comparable between the 2 groups, but the median duration of morning stiffness was twice as long in the placebo group (60.0 minutes versus 30.0 minutes in the prednisolone group).

The study medication was well tolerated. Two adverse events were reported in the placebo group. In 1 patient, a skin portal infection of the wrist developed after the first arthroscopic procedure, leading to withdrawal of that patient from the study. In a second patient, portal inflammation of the knee developed after the first arthroscopic procedure, but that patient completed the study. One subject receiving prednisolone reported weight gain and bruising.

Clinical response.

As expected, there was a marked positive effect of prednisolone treatment on the Disease Activity Score in 28 joints (DAS28) (24). After treatment, the mean DAS28 was 2.0 units lower (95% confidence interval [95% CI 1.0-3.0]) in patients who received prednisolone than in those who received placebo (Figure 1). The mean (±SD) DAS28 decreased from 6.27 ± 0.95 before prednisolone therapy to 4.11 ± 1.43 after therapy.
Table 1. Characteristics of the 21 study patients with active RA.*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Prednisolone Group (n=10)</th>
<th>Placebo Group (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>49.4 (32-63)</td>
<td>55.7 (37-69)</td>
</tr>
<tr>
<td>No. men/no. women</td>
<td>5/5</td>
<td>4/7</td>
</tr>
<tr>
<td>% currently receiving DMARDs</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>% rheumatoid factor positive</td>
<td>70</td>
<td>55</td>
</tr>
<tr>
<td>No. knee biopsy/no. wrist biopsy/no. ankle biopsy</td>
<td>7/2/1</td>
<td>8/3/0</td>
</tr>
<tr>
<td>ESR, mm/hour</td>
<td>29.8 (4-107)</td>
<td>27.4 (5-92)</td>
</tr>
<tr>
<td>CRP, mg/liter</td>
<td>12.5 (3-150)</td>
<td>16.3 (3-97)</td>
</tr>
<tr>
<td>DAS28 score</td>
<td>6.27 (4.59-7.98)</td>
<td>5.98 (4.56-7.59)</td>
</tr>
<tr>
<td>Disease duration, median months (range)</td>
<td>17.0 (2-134)</td>
<td>22.0 (3-168)</td>
</tr>
<tr>
<td>Morning stiffness, median minutes (range)</td>
<td>30.0 (0-330)</td>
<td>60.0 (0-960)#</td>
</tr>
<tr>
<td>Patient's assessment of pain in biopsied joint, mm (0-100 mm VAS)</td>
<td>48.5 (0-100)</td>
<td>47.3 (5-83)</td>
</tr>
<tr>
<td>Patient's assessment of pain, mm (0-100-mm VAS)</td>
<td>56.3 (0-96)</td>
<td>46.4 (16-78)</td>
</tr>
<tr>
<td>Patient's global assessment of disease activity, mm (0-100-mm VAS)</td>
<td>69.2 (49-100)</td>
<td>55.0 (15-100)</td>
</tr>
<tr>
<td>Physician's global assessment of disease activity, mm (0-100-mm VAS)</td>
<td>59.0 (37-76)</td>
<td>45.2 (9-76)</td>
</tr>
<tr>
<td>Tender joint count (0-28 scale)</td>
<td>12.9 (6-26)</td>
<td>13.0 (5-23)</td>
</tr>
<tr>
<td>Swollen joint count (0-28 scale)</td>
<td>12.6 (6-25)</td>
<td>10.7 (3-17)</td>
</tr>
<tr>
<td>HAQ score (0-3 scale)</td>
<td>1.92 (1.1-2.7)</td>
<td>1.92 (1.2-3.0)</td>
</tr>
</tbody>
</table>

* Except where indicated otherwise, values are the mean (range). RA = rheumatoid arthritis; DMARDs = disease-modifying antirheumatic drugs; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; DAS28 = Disease Activity Score in 28 joints; VAS = visual analog scale; HAQ = Health Assessment Questionnaire.

# 960 minutes indicates “all-day morning stiffness”.

This effect was not observed in the placebo group, in which the mean (±SD) DAS28 was 5.98 ± 0.99 before treatment versus 5.68 ± 1.31 after treatment. After 2 weeks of treatment, an ACR20 improvement response (25) was observed in 7 patients in the prednisolone group but in none of the patients in the placebo group. The ESR and the CRP level, respectively, were 69% lower (95% CI 54-79%) and 70% lower (95% CI 48-92%) after prednisolone treatment than after placebo treatment. For the ESR, the geometric mean declined from 29.81 mm/hour...
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(coefficient of variation [CV] 123.89%) before prednisolone treatment to 10.29 mm/hour (CV 118.67%) after treatment. For the CRP level, the geometric mean declined from 12.53 mg/liter (CV 181.35%) before prednisolone treatment to 4.34 mg/liter (CV 92.38%) after treatment.

Figure 1. Effect of treatment with either prednisolone or placebo on the individual Disease Activity Score in 28 joints (DAS28). A marked reduction was observed after 2 weeks of prednisolone treatment.

Serum cytokine levels were generally too low to be detected, with the exception of IL-18. Mean levels of IL-18 decreased from 219.0 ± 119.2 pg/ml before treatment to 179.7 ± 80.1 pg/ml after treatment in the prednisolone group. In the placebo group, the mean level decreased from 181.9 ± 121.5 pg/ml to 170.2 ± 106.4 pg/ml. These data were not formally analyzed.

The level of serum MMP-3 was, on average, 1.8-fold higher following prednisolone therapy compared with placebo therapy; however, the 95% CI was wide (0.76-4.22). For urine markers, there was a trend toward a reduction in urinary pyridinoline, deoxypyridinoline, and markers of bone degradation corrected for creatinine measurements; however the 95% CIs included the value for no effect, possibly due to the relatively small numbers of patients.

**Immunohistochemical findings.**

Synovial tissue from 19 of the 20 patients who completed the study could be analyzed before treatment and after 2 weeks of treatment. One synovial tissue sample did not contain an intimal lining layer and was omitted from analysis. The results of this analysis are shown in Table 2. For macrophages, the estimated effect of prednisolone was large. Patients receiving prednisolone had fewer (mean 628 cells/mm² [95% CI 328-927]) macrophages after therapy compared with those receiving placebo. In the prednisolone group, the total number of CD68+ cells decreased from 1,038 ± 283 cells/mm² before treatment to 533 ± 248 cells/mm² after treatment, whereas in the placebo group there was an increase from 836 ± 408 cells/mm² to 1,052 ± 422 cells/mm². This effect of prednisolone was mainly attributable to a decrease in the number of macrophages localized in the synovial sublining. A representative picture of
CD68 staining before and after treatment is shown in Figure 2A. Graphs showing the effect of treatment on CD68+ sublining macrophages and total CD68+ macrophages are shown in Figures 2B and C, respectively.

Table 2. Results of immunohistochemical analysis of synovial tissue obtained from patients with rheumatoid arthritis.

<table>
<thead>
<tr>
<th></th>
<th>Prednisolone</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD68 Total</strong></td>
<td>1,038 ±283</td>
<td>836 ±408</td>
</tr>
<tr>
<td><strong>CD68 Lining</strong></td>
<td>1,144 ±88</td>
<td>94 ±92</td>
</tr>
<tr>
<td><strong>CD68 Sublining</strong></td>
<td>930 ±285</td>
<td>765 ±405</td>
</tr>
<tr>
<td><strong>CD163</strong></td>
<td>1,559 ±451</td>
<td>1,369 ±797</td>
</tr>
<tr>
<td><strong>CD14</strong></td>
<td>203 (191)</td>
<td>157 (510)</td>
</tr>
<tr>
<td><strong>CD13</strong></td>
<td>381 (90)</td>
<td>299 (378)</td>
</tr>
<tr>
<td><strong>CD4</strong></td>
<td>360 (171)</td>
<td>243 (248)</td>
</tr>
<tr>
<td><strong>CD68</strong></td>
<td>1,937 (934)</td>
<td>1,909 (784)</td>
</tr>
<tr>
<td><strong>CD22</strong></td>
<td>11 (372)</td>
<td>5 (709)</td>
</tr>
<tr>
<td><strong>CD38</strong></td>
<td>37 (1,936)</td>
<td>128 (350)</td>
</tr>
<tr>
<td><strong>CD138</strong></td>
<td>36 (240)</td>
<td>45 (438)</td>
</tr>
<tr>
<td><strong>CD163</strong></td>
<td>215 (167)</td>
<td>245 (125)</td>
</tr>
<tr>
<td><strong>CD4</strong></td>
<td>212 (231)</td>
<td>128 (331)</td>
</tr>
<tr>
<td><strong>CD8</strong></td>
<td>48 (143)</td>
<td>29 (82)</td>
</tr>
<tr>
<td><strong>Granzyme B</strong></td>
<td>1,031 (208)</td>
<td>1,020 (894)</td>
</tr>
<tr>
<td><strong>CD55</strong></td>
<td>1,041 (64)</td>
<td>834 (61)</td>
</tr>
<tr>
<td><strong>CCR1</strong></td>
<td>10,20 (244)</td>
<td>21,980 (181)</td>
</tr>
<tr>
<td><strong>TNF</strong></td>
<td>177,050 (56)</td>
<td>172,152 (85)</td>
</tr>
<tr>
<td><strong>IL-1α</strong></td>
<td>124,739 (135)</td>
<td>121,322 (82)</td>
</tr>
<tr>
<td><strong>IL-1β</strong></td>
<td>248,882 (52)</td>
<td>239,060 (22)</td>
</tr>
<tr>
<td><strong>MMP-1</strong></td>
<td>361 (6,621)</td>
<td>267 (2,913)</td>
</tr>
<tr>
<td><strong>MMP-13</strong></td>
<td>421 (78)</td>
<td>322 (79)</td>
</tr>
<tr>
<td><strong>αvβ3</strong></td>
<td>20,728 (270)</td>
<td>9,443 (171)</td>
</tr>
<tr>
<td><strong>bFGF</strong></td>
<td>5,637 (414)</td>
<td>5,679 (383)</td>
</tr>
<tr>
<td><strong>VEGF</strong></td>
<td>47,895 ±25,060</td>
<td>32,000 ±24,867</td>
</tr>
<tr>
<td><strong>VWF+ vessels</strong></td>
<td>65 ±17</td>
<td>53 ±13</td>
</tr>
</tbody>
</table>

Values for CD68+ macrophages and CD32+ cells (Fcγ receptor II) are the mean ± SD cells/mm². Values for CD163+ macrophages (overall), CD14+ cells (monocytes), CD64+ cells (Fcγ receptor I), CD5+ lymphocytes (B and T cells), CD22+ lymphocytes (B cells), CD38+ cells (plasma cells), CD138+ cells (plasma cells), CD3+ (T cells), CD4+ and CD8+ T cells, granzyme B+ cells, CCR1+ cells (chemokine receptor), and CD55+ fibroblast-like synoviocytes are the geometric mean (coefficient of variation [CV] [%]) positive cells/mm². Values for tumor necrosis factor-α (TNF-α), interleukin-1α (IL-1α), IL-1β, matrix metalloproteinase 1 (MMP-1), MMP-13, αvβ3, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and von Willebrand's factor (vWF+) vessels are the geometric mean (% CV) or the mean ± SD integrated optical density.

Figure 2. A. Representative photomicrograph showing CD68+ macrophages (reddish-brown staining) in rheumatoid synovial tissue before and after treatment with prednisolone or placebo (original magnification × 200). A marked reduction in the number of CD68+ macrophages was observed after 2 weeks of prednisolone treatment. B. Graph showing the effect of treatment with prednisolone on sublining (sl) CD68+ macrophages.
marked reduction was observed after 2 weeks of prednisolone treatment for 2 weeks. C, Graph showing the effect of treatment with prednisolone on total CD68+ macrophages. A marked reduction was observed after 2 weeks of prednisolone treatment. A full colour image of figure 2A is provided in the Appendix (Chapter 5, figure 2A).

A.

Prednisolone

Placebo

Pre

Post

B.

Prednisolone Pre  Prednisolone Post  Placebo Pre  Placebo Post
There was also a decrease following prednisolone treatment in the number of CD5+ stained cells (B cells and T cells), CD163+ stained cells (macrophages), and CD4+ stained cells, accompanied by increases in the placebo group, combining to result in a large difference between the treatment groups in these markers. There were clear trends toward decreased infiltration by CD38+ plasma cells and CD55+ fibroblast-like synoviocytes after active treatment, but CIs included the value for no treatment effect.

Staining for cytokines showed that expression of IL-1β was 40% lower (95% CI 18-56%) following prednisolone therapy compared with placebo (the geometric mean integrated optical density [IOD] decreased from 248.882 [CV 52%] to 147,428 [CV 39%] following prednisolone treatment), and expression of TNF-α was 52% lower (95% CI 10-74%) following prednisolone therapy versus placebo (the geometric mean IOD decreased from 177,050 [CV 56%] to 87,855 [CV 95%] after prednisolone treatment). This effect was mainly attributable to changes in the synovial sublining (Figure 3).

A trend toward decreased vascularity was observed in the prednisolone-treated group for the markers of angiogenesis studied, especially in αβ3+ newly formed blood vessels (change in geometric mean IOD from 20.728 [CV 270%] to 7.966 [CV 179%]). This was 60% lower (95% CI 84% lower to 3% higher) after prednisolone therapy compared with placebo.

For all other markers analyzed, estimates for the size of treatment effect varied widely, with many of them showing a trend toward a reduction in the prednisolone group; however, CIs included the value for no effect.

DISCUSSION

This study was conducted to address the question of which features in RA synovial tissue samples could be used as a biomarker for clinical efficacy in relatively small studies of short duration. The results presented here show that clinically effective prednisolone therapy (the
COBRA regimen) is particularly associated with a marked reduction in macrophage infiltration in the synovial tissue from RA patients after 2 weeks of treatment.

**Figure 3.** Representative photomicrograph showing tumor necrosis factor α (TNF-α) expression (reddish-brown staining) in rheumatoid synovial tissue before and after treatment with prednisolone or placebo (original magnification x 200). A strong decrease in TNFα expression was observed after 2 weeks of prednisolone treatment. A full colour image of figure 3 is provided in the Appendix (Chapter 5, figure 3).

Results of a previous study suggested a decrease in T cell numbers after at least 6 months of treatment with gold; macrophages were not analyzed in that study (26). Another study that included the evaluation of macrophages showed a clear reduction in macrophages 12 weeks after gold therapy (27). The expression of macrophage-derived cytokines such as IL-1β, TNF-α, and IL-6 was also found to be reduced (27). Similarly, 16 weeks of treatment with methotrexate led to a decrease in the numbers of various inflammatory cells, including T cells, plasma cells, and macrophages (15:28). The expression of macrophage-derived cytokines, adhesion molecules, and MMPs was also reduced after methotrexate treatment (15:28:29). Similar effects were observed after effective treatment with leflunomide (15).

Consistent with the results from the present study after 2 weeks of corticosteroid treatment, a marked reduction in the number of synovial macrophages was reported in patients who achieved clinical remission, as defined by the ACR response criteria, after 3 months of treatment with various DMARDs (30). Treatment with biologic agents and targeted small molecules, including infliximab (31-33), etanercept (34), anakinra (8), and a specific CCR1 antagonist (35), also resulted in decreased synovial cellularity. Results of these studies support the notion that there is a possible relationship between changes in macrophage numbers and both expression of macrophage-derived cytokines and clinical improvement. Of interest, treatment with infliximab induced a significant reduction in macrophage numbers only 48 hours after the first infusion of the antibody, especially in those patients who fulfilled the European League Against Rheumatism response criteria 1 month after initiation of anti-TNF therapy (36). The change in sublining macrophage numbers was far more pronounced than changes in other cell types.

Taken together, the experience with both DMARDs and targeted therapies generally suggests that, although the mechanism of action may differ among different therapeutic strategies, the ultimate effect associated with clinical improvement is a reduction in the numbers of macrophages and the expression of macrophage-derived cytokines in the synovium. Consistent with this concept, stepwise multiple regression analysis revealed that these same
biomarkers were associated with scores for local disease activity in RA in a cross-sectional study (37). The present study is the first placebo-controlled trial using a known clinically effective therapy designed to identify synovial tissue biomarkers associated with clinical efficacy after short-term treatment in a relatively small number of patients. Consistent with suggestions from previous studies described above, the ANCOVA model confirmed that the number of macrophages in the synovial sublining could be used as a biomarker for clinical efficacy.

The changes in the synovium might be explained in part by changes in cell migration attributable to reduced expression of adhesion molecules and chemokines, as suggested previously (38-40). In addition, the results from this study indicate that inhibition of (neo)angiogenesis could play a role as well. There was a trend toward reduced expression of various markers of angiogenesis (VEGF, bFGF, and αvβ3). Corticosteroids may have direct effects on these factors (41;42). In addition, indirect effects due to inhibition of chemokine and cytokine expression could play a role (43). Although there were clear trends in the same direction for factors involved in angiogenesis, the 95% CIs included the value for no effect, possibly due to the relatively small number of patients.

There were also trends toward decreased infiltration by T cells, plasma cells, and fibroblast-like synoviocytes after active treatment. Conceivably, these changes would be more pronounced after more prolonged treatment, similar to observations in serial biopsy specimens obtained after treatment with various DMARDs (30). In addition, the expression of TNF-α and IL-1β was greatly reduced in rheumatoid synovial tissue after treatment with prednisolone compared with placebo, in accordance with previous studies (6;44). It should be noted that these cytokines are mainly produced by macrophages in the inflamed synovium.

In conclusion, prednisolone therapy is associated with a marked reduction in synovial tissue macrophage infiltration. These data lend further support to the hypothesis that synovial sublining layer macrophages represent the parameter most sensitive to change after effective treatment of RA and may be used as a biomarker when novel therapeutic agents for RA are screened for potential efficacy.

Reference List


Identification of Biomarkers in Synovial Tissue


(34) Verschueren PC, Markusse HM, Smeets TJM, Kraan MC, Breedveld FC, Tak PP. Reduced cellularity and expression of adhesion molecules and cytokines after treatment with soluble human recombinant TNF receptor (P75) in RA patients. Arthritis & Rheumatism. 1992;42.


