Successful treatment of rheumatoid arthritis is associated with a reduction in synovial membrane cytokines and cell adhesion molecule expression

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Abstract

Objective. To investigate the change in synovial membrane cytokine content and cell adhesion molecule expression in sequential biopsies from the same knee joint of patients with rheumatoid arthritis, before and following anti-rheumatic drug treatment and to assess the relationship of these changes with clinical responses to the drug treatment.

Methods. A selected group of patients with rheumatoid arthritis, some of whom had achieved a disease remission based on American College of Rheumatology (ACR) criteria, were included in this study. Sequential synovial biopsies obtained before and throughout the treatment period were studied by immunohistochemical labelling techniques for the cellular content, production of a range of pro- and anti-inflammatory cytokines and the expression of cell adhesion molecules. The staining was quantitated using computer-assisted digital image analysis.

Results. There was a decrease in tumour necrosis factor-α (TNFα) and interleukin-1β (IL-1β) production in the synovial membrane lining and sublining of all patients who responded to treatment. The changes in IL-1 receptor antagonist production were variable. Paradoxically, there was a trend to decreased synovial membrane production of the anti-inflammatory cytokines, IL-10 and transforming growth factor-β (TGFβ), while IL-4 was not detectable in any of the synovial membrane biopsies. A significant reduction in the density and total amount of E-selectin expression in the synovial membrane was seen. Similarly, intercellular adhesion molecule-1 (ICAM-1) expression in the lining and sublining was decreased in those patients who had a significant clinical response to drug treatment or attained disease remission. There were no consistent or significant changes seen in the expression of other cell adhesion molecules in the synovial membranes of these patients.

Conclusions. Successful drug treatment of rheumatoid arthritis patients is characterized at the synovial membrane level by a decrease in TNFα, IL-10 and TGFβ production. Some (E-selectin and ICAM-1) but not all (P-selectin, VCAM-1, PECAM-1) cell adhesion molecules are modulated in patients who respond clinically to drug treatment. E-selectin and ICAM-1 may be important targets for the development of future drug treatments for rheumatoid arthritis.

Key WORDS: Rheumatoid arthritis, Remission, Synovial membrane, Cytokines, Cell adhesion molecules.

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown aetiology characterized by a chronic synovitis which often results in joint destruction. The mechanisms responsible for the development of these lesions are largely unknown, with both the T lymphocyte and the macrophage being considered as the pivotal
cell involved in the pathogenesis of RA [1, 2]. It is, however, clear that pro- and anti-inflammatory cytokines, derived predominantly from macrophage lineage cells in the synovial membrane, play a major role in the initiation and perpetuation of the chronic inflammatory process in the RA synovial membrane [3, 4].

Previous studies of cytokines in the RA synovium have predominantly utilized synovial tissue obtained from patients at the time of joint replacement surgery, with the potential confounders of variable duration of disease, disease activity and previous drug treatments. Pro-inflammatory cytokines, such as interleukin-1α and β (IL-1α and IL-1β), tumour necrosis factor-α (TNFα), granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-6 are all produced by the RA synovial membrane [1, 3, 4]. There also appears to be a compensatory anti-inflammatory response in RA synovial membranes which includes the IL-1 receptor antagonist protein (IL-1Ra) [5] p55 and p75 soluble TNF receptors (sTNF-r) [6, 7], IL-4 [3], IL-10 [3] and transforming growth factor-β (TGFβ) [8].

The establishment of a chronic synovitis involves the traffic of circulating inflammatory cells into and through the synovial membrane [9, 10], regulated by cell adhesion molecules which are in turn regulated by pro-inflammatory cytokines. Manipulation of the expression of cell adhesion molecules can have a profound effect on the inflammatory process in RA, as suggested in a study of anti-intercellular adhesion molecule (anti-ICAM-1) treatment in refractory RA patients [11].

Disease-modifying anti-rheumatic drugs (DMARDs), including gold and methotrexate, are the main therapeutic agents available at present for the treatment of RA patients, yet their mechanisms of action are largely unknown. There have been few studies examining prospectively the effect of drug treatment on synovial membrane cytokine content or cell adhesion molecule expression. Two studies have examined the effect of intramuscular sodium aurothiomalate on the cytokine content of sequential synovial membrane biopsies from RA patients [12, 13] and a further study demonstrated a reduction in ELAM-1 (E-selectin) with intramuscular gold treatment [14]. These studies all used a blind percutaneous needle biopsy technique to obtain synovial membrane biopsies. This technique is limited by access to synovial tissue only from the suprapatellar pouch, along with the relatively high failure rate in providing adequate synovial tissue for study [12–14]. This can be a major difficulty in research studies involving sequential sampling of the synovium before and after therapeutic intervention. In addition, the follow-up of these studies was relatively short and there was no attempt to correlate the changes in synovial membrane cytokines or cell adhesion molecule expression with the disease status of the individual patient (remission, controlled disease, active disease). We have recently demonstrated the feasibility of performing serial arthroscopic synovial biopsies on the same knee joint to obtain synovial membrane samples in RA patients [15] and have used this technique to demonstrate the effect of intravenous corticosteroid ‘pulse’ treatment on cytokine and cell adhesion molecule expression at the synovial membrane level [16, 17]. We have also recently demonstrated that successful DMARD treatment of RA patients has a substantial effect on macrophage content of the synovial membrane, with a less marked effect on memory T cell content [18].

Recent clinical trials have demonstrated the successful targeting of specific cytokines, TNFα and IL-1β, in the treatment of RA [19–21], raising the question as to whether successful treatment with DMARDs also modifies similar or different biological mediators. This study examined the effect of DMARD treatment on synovial membrane cytokine and cell adhesion molecule expression in RA patients.

Methods

Reagents

Ethanol, methanol, diaminobenzidine, methyl green, haematoxylin, xylene, sodium chloride and DPX (non-aqueous mounting medium) were obtained from BDH Laboratories (Poole, UK). Hydrogen peroxide was obtained from Univar (Auburn, Sydney, Australia). Normal donkey serum and biotinylated donkey antimouse secondary antibody were obtained from Jackson Immunoresearch (Westgrove, PA, USA). Avidin–biotin–horseradish peroxidase complex (Vectastain kit) was obtained from Vector Laboratories (Burlingame, CA, USA).

Patients

The patients in this study are described in Table 1. All fulfilled the 1987 American College of Rheumatology (ACR) criteria for RA [22], and were selected from a larger group of 40 RA patients being followed with sequential synovial biopsies after the initiation of DMARD treatment.

Patient assessment

Clinical assessment was performed using visual analogue scores (measured on a 100 mm horizontal scale anchored at both ends) for pain, generalized stiffness, patient and physician global scores, as well as a health assessment questionnaire (HAQ). In addition, a tender and swollen joint count was performed. Serum C-reactive protein (CRP) was used as a laboratory assessment of inflammation. Response to treatment was assigned utilizing the ACR criteria for improvement [23] and remission [24]. ‘Shared’ epitope, the common HLA-DRB1 allele in RA patients, was measured by flow cytometry [25], using a commercial kit (Terra Nova Biotechnology, St John’s, Newfoundland, Canada). All patients had annual X-rays of their hands and feet. These X-rays were assessed by two radiologists, blind to treatment outcomes, using the Larsen et al. [26] and Sharp et al. [27] methods of evaluation.
Table 1. Patient demographic details

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Duration of disease (yr)</th>
<th>Rheumatoid factor (U/ml)</th>
<th>Shared epitope status</th>
<th>Drug treatment</th>
<th>Change in Larsen score</th>
<th>Change in erosion score</th>
<th>Response to treatment</th>
</tr>
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<tr>
<td>RA1</td>
<td>65</td>
<td>M</td>
<td>0.75</td>
<td>263</td>
<td>Positive</td>
<td>IM gold/MTX</td>
<td>0</td>
<td>+2</td>
<td>Remission</td>
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<tr>
<td>RA2</td>
<td>60</td>
<td>M</td>
<td>25</td>
<td>2090</td>
<td>Positive</td>
<td>IM gold</td>
<td>+2</td>
<td>−3</td>
<td>ACR 90%</td>
</tr>
<tr>
<td>RA3</td>
<td>77</td>
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<td>20</td>
<td>349</td>
<td>Positive</td>
<td>IM gold</td>
<td>+6</td>
<td>+1</td>
<td>Remission</td>
</tr>
<tr>
<td>RA4</td>
<td>71</td>
<td>F</td>
<td>18</td>
<td>335</td>
<td>Negative</td>
<td>IM gold</td>
<td>+1</td>
<td>−2</td>
<td>Remission</td>
</tr>
<tr>
<td>RA5</td>
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<td>M</td>
<td>24</td>
<td>501</td>
<td>Positive</td>
<td>MTX</td>
<td>+18</td>
<td>+20</td>
<td>ACR 20%</td>
</tr>
<tr>
<td>RA6</td>
<td>83</td>
<td>F</td>
<td>2</td>
<td>&lt;20</td>
<td>Negative</td>
<td>MTX</td>
<td>0</td>
<td>−2</td>
<td>Remission</td>
</tr>
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<td>F</td>
<td>0.5</td>
<td>569</td>
<td>Positive</td>
<td>MTX</td>
<td>+1</td>
<td>−2</td>
<td>Remission</td>
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<td>F</td>
<td>10</td>
<td>&lt;20</td>
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<td>MTX</td>
<td>+2</td>
<td>−1</td>
<td>ACR 80%</td>
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<tr>
<td>RA9</td>
<td>76</td>
<td>M</td>
<td>0.25</td>
<td>43</td>
<td>Negative</td>
<td>Sulphasalazine</td>
<td>+1</td>
<td>+2</td>
<td>Remission</td>
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<tr>
<td>RA10</td>
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<td>588</td>
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<td>MTX</td>
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<td>+10</td>
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<tr>
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<td>0.5</td>
<td>1670</td>
<td>Positive</td>
<td>IM gold/MTX</td>
<td>+6</td>
<td>0</td>
<td>ACR 50%</td>
</tr>
<tr>
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<td>75</td>
<td>M</td>
<td>0.5</td>
<td>&lt;20</td>
<td>Negative</td>
<td>MTX</td>
<td>+2</td>
<td>+2</td>
<td>ACR 90%</td>
</tr>
<tr>
<td>RA13</td>
<td>75</td>
<td>M</td>
<td>18</td>
<td>335</td>
<td>Negative</td>
<td>IM gold</td>
<td>+4</td>
<td>+6</td>
<td>Remission</td>
</tr>
</tbody>
</table>

MTX, methotrexate; IM gold, intramuscular sodium aurothiomalate.

Arthoscopic biopsies

Synovial membrane samples (minimum of eight biopsies) were obtained from the same knee joint from each patient, before and at 3- or 6-monthly intervals after commencing drug treatment, under direct vision using a 2.7 mm needle arthroscope (Dyonics, Andover, MA, USA) and standard approaches as previously described [15–17]. Biopsies were taken from regions throughout the knee joint, including areas close to the cartilage–pannus junction, and were processed together as a single block of tissue. The site of each synovial biopsy was documented on a knee diagram and subsequently synovial biopsies in the same patient were taken from similar regions within the knee joint.

Synovial tissue was immediately processed for frozen and paraffin sections as previously described [16, 17]. As formalin-fixed, paraffin-embedded sections had superior preservation of architecture, compared with frozen sections, the former were used for all immunohistochemical staining except for the detection of cell adhesion molecules which required frozen sections. All frozen tissues were cut to 6 µm thickness on the same cryostat at the same time, while all paraffin sections were cut to 4 µm thickness on the same microtome at the same time.

This project was approved by an institutional ethics committee and informed consent was obtained from each patient.

Monoclonal antibodies

Anti-TNFα antibody (IgG1) was supplied by Monosan (Uden, The Netherlands), anti-IL-1Ra antibody (monoclonal antibody 1.4, IgG1) was supplied by Upjohn (Kalamazoo, USA), anti-IL-1β antibody (monoclonal antibody 12E9, IgM) was supplied by Oncogene Science (Manhasset, NY, USA) and anti-IL-4 (IgG1) was supplied by Genzyme Diagnostics (Cambridge, MA, USA). Anti-E-selectin antibody (CD62E, IgG1), anti-ICAM-1 antibody (CD54, IgG1), anti-P-selectin antibody (CD62P, IgG1), anti-PECAM-1 (platelet-endothelial cell adhesion molecule) (CD31, IgG1) and anti-VCAM-1 (vascular cell adhesion molecule) antibody (IgG1) were supplied by Novoceastra Laboratories (Newcastle upon Tyne, UK). All the above antibodies used for immunochemistry were mouse monoclonals which have been conclusively demonstrated to be specific for the target cytokine or cell adhesion molecule by the manufacturers. Ulex Europaeus (UEA-1), a marker of endothelium, was obtained from Vector Laboratories. In addition, two polyclonal anti-cytokine antibodies were used—anti-IL-10 (polyclonal sheep antibody) from Genzyme Diagnostics and anti-TGFβ (polyclonal goat antibody) from R & D Systems (Abingdon, UK).

The isotype-specific negative controls X63 (IgG1, recognizes a mouse myeloma protein) and FMC41 (IgM, recognizes blood group A-associated antigen) were all generous gifts from Professor Heddy Zola and have been described previously [16, 17].

Tissue processing and immunoperoxidase staining

Immunohistochemical staining was performed as previously described [16, 17, 28, 29], using monoclonal antibodies to characterize specific cell populations. Staining of all synovial membrane biopsies for a particular cytokine or cell adhesion molecule was performed at the same time, to avoid day to day variation of the intensity of the immunohistochemical staining. Negative controls were performed using irrelevant isotype control antibodies, normal donkey, sheep or goat serum alone or by leaving out the secondary antibody. A positive control (either lymph node or synovial tissue with known staining characteristics) was used in each run.

Colour video image analysis

The immunostained sections were examined using a computer-assisted colour video image analysis system,
previously validated and described for synovial membranes [16, 17, 29]. At least two biopsies from any single time point were analysed, with a minimum of six high power fields per time point. Thresholds were set for each section to detect and measure the identified reaction product (cytokine or cell adhesion molecule). Once a threshold was chosen, this was kept constant for all measurements on synovial membrane sections from the same patient. All sections from any single patient for each cytokine or cell adhesion molecule were analysed in the same sitting. The measurements made included the mean optical density (MOD) field, which is a measure of the average cellular concentration of cytokine or cell adhesion molecule, derived by dividing the integrated optical density (IOD) by the area of staining and the IOD, which is proportional to the total amount of cytokine or cell adhesion molecule. The repeatability of measurements was within 10% [29], which was mainly due to observer variability in field selection. All sections were measured by one blind observer (MS) who was unaware of the order of biopsies from any one patient.

In addition, between 200 and 900 vessels (usually all the vessels in a section) were counted by a single observer (MS) who was blind to the order of biopsies from any single patient. A score for the number of positive vessels as a percentage ratio of the total number of vessels was calculated in the following way: number of positive vessels

\[
\frac{\text{number of PECAM-1- or UEA-1-positive vessels}}{3} \times 100\%.
\]

Also, a score was given for the amount of vessel wall stained: 0 = no staining (0%); + = 1–25%; ++ = 26–50%; +++ = 51–75%; ++++ = >75% of vessel wall stained.

Statistical analysis

The results are given as mean ± standard deviation (S.D.). To allow for a correlation between observations and to analyse the data longitudinally, the statistical approach of generalized estimating equations [30] was used to compare the clinical response data at various time points for each patient. In addition, Student t-tests were used to compare the change in the group mean for observations at different time points. Differences were considered to be significant at \( P < 0.05 \).
Sequential synovial biopsies from RA6, who achieved a clinical remission after oral methotrexate treatment, taken at (A) initiation of treatment, (B) 6 months, (C) 12 months and (D) 18 months after commencing treatment. Sections were immunostained with an antibody specific for TNFα. There was a substantial decrease in the density and total amount of TNFα in the lining and sublining of the synovial membrane with treatment.
Results

All patients fulfilled ACR criteria for RA. Disease duration was a mean of 10.8 yr (range 3 months to 25 yr) with nine of the 13 patients demonstrating joint erosions on entry into the study (Table 1). Seven of the 13 patients were positive for the shared epitope and only two patients did not express the shared epitope and had no joint erosions on X-rays of the hands and feet.

There was a good clinical response to treatment in 11 of the 13 patients who were included in this study (Table 2), with seven achieving disease remission (intramuscular gold three, oral methotrexate two, intramuscular gold and oral methotrexate one, sulphalazine one), four achieving an ACR 50% or greater response (oral methotrexate two, intramuscular gold one, intramuscular gold and oral methotrexate one), while one patient (oral methotrexate) only achieved an ACR 20% response and one had no response to oral methotrexate. There was a significant reduction in both serum CRP and rheumatoid factor levels in response to treatment (Fig. 1).

Cellular infiltrate in synovial membranes

The effect of DMARD treatment on synovial membrane cellular content in this group of patients has been recently published [18]. The two patients with minimal response to DMARD treatment (RA5 and RA10) did not demonstrate a significant change in macrophage content of the synovial membrane with treatment.

Synovial membrane cytokine profiles

There was a dramatic reduction in TNFα production (Fig. 2), both in the lining and sublining of the synovial membrane, especially in those patients who achieved at least an ACR 50% response or remission with treatment (Fig. 3). Although there was also a reduction in synovial membrane IL-1β content in both the lining and sublining (Fig. 4), these changes were not as pronounced or as consistent as those changes seen in TNFα content in the same patient.

**Fig. 3.** Change in the total amount (IOD, top panel) and density (MOD, bottom panel) of TNFα expression in the lining and sublining regions of the synovial membrane as a result of DMARD treatment. The reduction in synovial membrane TNFα is predominantly seen in those patients who achieved a clinical remission. Error bars define the s.d. for each measurement. *P < 0.05 compared with baseline values.

**Fig. 4.** Changes in IL-1β and IL-1Ra content in the lining and sublining regions of the synovial membrane as a result of DMARD treatment. The reduction in synovial membrane IL-1β is generally much less than that seen with TNFα, even in those patients who achieved a clinical remission, while there is no consistent change in IL-1Ra content with treatment. There was no significant change in density (MOD) of IL-1β or IL-1Ra in the synovial membrane with treatment. Error bars define the s.d. for each measurement. *P < 0.05 compared with baseline values.
Changes in the synovial membrane content of IL-1Ra with treatment were inconsistent, with some patients showing an increase while others showed no change (Fig. 4).

It was expected that there would be a corresponding increase in anti-inflammatory cytokine content in the synovial membranes of RA patients responding to treatment, but the anti-inflammatory cytokines IL-10 and TGFβ (Fig. 5) either showed little change or a marked decrease in response to anti-rheumatic drug treatment. IL-4 could not be detected in any of the synovial membrane biopsies, irrespective of disease activity, response to treatment or anti-rheumatic drug used for treatment. There was no change in the cytokine content of the sequential synovial biopsies from patients who failed to achieve an ACR response greater than 20% (RA5 and RA10, results not shown).

There was no correlation with initial disease activity, ‘shared’ epitope status or anti-rheumatic drug used for treatment and changes in synovial membrane cytokine content.

Synovial membrane cell adhesion molecule expression

There was a marked decrease in E-selectin (Figs 6A–D and 7) and ICAM-1 (Fig. 6E–H) expression in the synovial membrane of RA patients who responded clinically to drug treatment, with little change in those RA patients who had a minimal response to treatment (results not shown). This was statistically significant for E-selectin density and total amount (Fig. 8) and ICAM-1 in the lining layer and sublining regions (Figs 6, 7). Similar results were seen when the percentage of blood vessels expressing E-selectin was determined, but no significant change in the percentage of blood vessels expressing other cell adhesion molecules was seen as a result of drug treatment (results not shown).

No significant change in the expression of P-selectin or PECAM-1 was seen in any of the RA patients, irrespective of the clinical response to drug treatment (results not shown). There was, however, a significant decrease in VCAM-1 expression in the synovial lining layer in some patients who attained an ACR 50% or greater response, but no significant change in the weak expression of VCAM-1 in sublining regions of the synovial membrane or on blood vessels (Figs 8, 9).

Discussion

Both animal models and clinical studies of RA have implicated TNFα and IL-1 in the pathogenesis of RA and suggest that the balance between cytokines and their natural antagonists is important in modulating the inflammatory response in RA [3, 7, 31]. Although TGFβ has potent anti-inflammatory properties, experimental animal models examining treatment with TGFβ have produced conflicting results [8].

Two further cytokines, IL-4 and IL-10, have biological actions which suggest that they may have major roles as anti-inflammatory cytokines [32–34]. IL-4 is known to suppress the production of pro-inflammatory cytokines such as IL-1, TNFα, IL-6, IL-8 and GM-CSF by monocytes and this appears to be at both transcriptional and post-transcriptional levels. It has also been shown that IL-4 will increase IL-1Ra production at the level of transcription. There is little or no IL-4 detectable at the mRNA or protein level in the synovial fluid of patients with RA [32]. Although in vitro experiments and studies of animal and ex vivo models of RA suggest that IL-4 has a potential anti-inflammatory role [33], our studies raise doubts as to whether IL-4 is capable of modulating the chronic inflammatory response [35, 36]. We were unable to detect any IL-4 in the synovial membranes of our patients, irrespective of the order of biopsy, disease activity or treatment effects. This suggests that, although IL-4 has a potential anti-inflammatory role in chronic inflammation, there is no evidence to support such a role in RA, at least in relation to successful treatment with currently utilized anti-rheumatic drugs.
FIG. 6. Sequential synovial biopsies from RA1, who achieved a clinical remission after intramuscular gold treatment, taken at initiation of treatment [(A) and (E)], 3 months [(B) and (F)], 6 months [(C) and (G)] and 12 months [(D) and (H)] after commencing treatment. Sections were immunostained with an antibody specific for E-selectin [(A)–(D)] (×132) or ICAM-1 [(E)–(H)] (×66). There was a substantial decrease in the density and total amount of E-selectin expression on endothelial cells and ICAM-1 expression on the synovial lining cells, with continued expression of ICAM-1 on endothelial cells.
Similarly, IL-10 has been shown to inhibit the production of pro-inflammatory cytokines by monocytes as well as inhibiting its own production, all occurring at the level of transcription. While IL-10 is undetectable in the synovial fluid, it is clearly present in RA synovial membrane and is detectable in macrophages and T cells isolated from RA synovial tissue [34]. The ability of IL-10 to inhibit antigen-specific T-cell proliferation by reducing major histocompatibility complex (MHC) class II expression and its inhibitory effects on pro-inflammatory cytokine secretion suggest that it may have a role as a suppressor of immune and inflammatory responses in RA [34, 36]. Therefore, modulation of IL-10 production in RA synovial tissue could have a significant effect on the chronic inflammatory process and may be a potential therapeutic target both for existing and new treatments for RA. However, there is abundant IL-10 production in the RA synovial membrane in patients with active disease, questioning the effectiveness of IL-10 as an anti-inflammatory cytokine. In response to intramuscular gold and oral methotrexate, IL-10 production at the synovial membrane level is decreased rather than increased as patients achieve a clinical response, suggesting that the major effect of these two anti-rheumatic drugs is to target macrophages [18], thereby decreasing the production of pro- and anti-inflammatory cytokines. This is supported by the decrease in synovial membrane IL-1β, TNFα, and TGFβ in response to anti-rheumatic drug treatment. IL-1Ra is not similarly affected, possibly as a result of alternative cellular sources, e.g. fibroblasts, which may not be affected by anti-rheumatic drug treatment. Similar results to those reported in this study have been demonstrated after methotrexate [37] and interferon-β [38] treatment, although biopsies were only performed up to 3 months after commencing treatment in these studies and it is not clear whether any of the patients included in these studies attained an ACR-defined remission. A study of RA patients treated with
an anti-TNFα antibody reported a modest reduction in IL-8 and monocyte chemotactic protein expression in the synovial membrane 2 weeks after treatment, but did not assess treatment effects on TNFα, IL-1α or β, IL-4, IL-10 or TGFβ [39]. Anti-CD4 treatment of RA patients, which had no effect on clinical disease activity, also had no significant effect on the synovial membrane content of a range of cytokines, including TNFα, IL-1β and IL-1Ra [40].

The expression of the endothelial selectins and ICAM-1 is transiently increased in vitro by a variety of pro-inflammatory mediators [9, 10]. However, in chronic inflammatory disorders such as RA, their expression is prolonged [17, 41–43], probably due to
the continued presence of pro-inflammatory mediators such as TNFα which are expressed at high levels in the synovium. PECAM-1 expression is not quantitatively regulated by pro-inflammatory mediators, although exposure to TNFα may redistribute PECAM-1 to cell junctions in some organs [44].

Both E- and P-selectin (CD62E and CD62P, respectively) are expressed on RA synovial endothelium [17, 41–43, 45, 46]. However, only the expression of E-selectin and not P-selectin is increased in RA when compared with osteoarthritis or normal synovium [41–43]. ICAM-1 is widely expressed in the synovial membrane of RA patients, including the vascular endothelium, synovial lining macrophages and fibroblasts as well as infiltrating leucocytes on which it is expressed at higher levels in RA when compared with normal synovium [41–43]. PECAM-1 is expressed on normal as well as RA synovial vascular endothelial cells and is not up-regulated at this site in RA compared with normal synovium [41, 47].

This study has demonstrated that the expression of some, but not all, cell adhesion molecules is modulated in the synovial membrane of RA patients who respond clinically to treatment with DMARDs, specifically methotrexate and intramuscular gold. As demonstrated previously with intramuscular gold [14] and, more recently, with intravenous ‘pulse’ corticosteroids [17], methotrexate [37], anti-CD4 antibodies [40], anti-TNFα antibodies [48] and IL-1Ra treatment [49], the main cell adhesion molecules which are down-regulated in response to drug treatment are E-selectin, VCAM-1 and ICAM-1, with little if any change in other cell adhesion molecules, particularly P-selectin, which has been demonstrated in vitro to have a major role in cell trafficking [9, 10]. The most marked reduction in the expression of these cell adhesion molecules was seen in those patients who achieved remission, as defined by ACR criteria [24]. There was little expression of VCAM-1 on sublining blood vessels, with no significant change with treatment, but there was a significant reduction in VCAM-1 expression by the synovial lining in some, but not all patients, probably as a result of changes in cytokine expression in the synovial membrane due to anti-rheumatic drug treatment.

This study demonstrates that it is not always possible to extrapolate the results of in vitro studies to the in vivo situation and also suggests that the future development of drug treatments for RA should focus on treatments which modulate or alter the function of specific cell adhesion molecules, particularly E-selectin and ICAM-1. There is clearly a hierarchy of pro-inflammatory cytokines in RA, with the predominant pro-inflammatory cytokine affected by anti-rheumatic drug treatment being TNFα. This is in agreement with the demonstrated efficacy of anti-TNF therapies in the treatment of RA patients [19, 20]. It remains to be demonstrated whether a prolonged remission induced by anti-rheumatic drug treatment will lead to a further reduction in synovial membrane IL-1β production and whether this will be reflected in the slowing or prevention of radiological disease progression. We have not been able to demonstrate any consistent effect of DMARD treatment on synovial membrane IL-1Ra production, despite the demonstration of reduced radiological progression of RA patients treated with recombinant human IL-1Ra [50]. Further longitudinal studies are now being undertaken in patients with early, non-erosive disease to address these questions.

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