ARTHRITIS & RHEUMATISM
Vol. 43, No. 8, August 2000, pp 1820–1830

MODULATION OF INFLAMMATION AND METALLOPROTEINASE EXPRESSION IN SYNOVIAL TISSUE BY LEFLUNOMIDE AND METHOTREXATE IN PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS

Findings in a Prospective, Randomized, Double-Blind, Parallel-Design Clinical Trial in Thirty-Nine Patients at Two Centers

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Objective. **Leflunomide and methotrexate have proven to be efficacious in reducing joint inflammation and slowing destruction in clinical trials of patients with rheumatoid arthritis (RA). This study was conducted to provide more insight into the mechanism of action of these agents in synovial tissue.**

Methods. **In a 2-center, prospective, randomized, double-blind clinical trial, we compared leflunomide (20 mg/day, after a 3-day 100 mg/day loading dose) and methotrexate (increased stepwise to 15 mg/week) treatment in patients with active RA. Paired synovial tissue biopsy samples were obtained by knee arthroscopy at baseline and after 4 months of treatment. Frozen synovial tissue sections were stained for macrophages (CD68), T cells (CD3), adhesion molecules (intercellular adhesion molecule 1 [ICAM-1], vascular cell adhesion molecule 1 [VCAM-1]), cytokines (tumor necrosis factor α, interleukin-1β [IL-1β]), matrix metalloproteinase 1 (MMP-1), and tissue inhibitor of metalloproteinases 1 (TIMP-1).**

Results. **Paired synovial tissue sections were available in 35 patients (16 taking leflunomide, 19 taking methotrexate). Both drugs displayed equal clinical efficacy, with 8 leflunomide-treated patients (50%) and 10 methotrexate-treated patients (53%) fulfilling the American College of Rheumatology 20% response criteria. Both compounds showed similar effects on synovial tissue: reduced numbers of macrophages and reduced ICAM-1 and VCAM-1 expression were noted after 4 months of treatment. Both leflunomide- and methotrexate-treated patients exhibited a decreased MMP-1:TIMP-1 ratio in the synovial tissue. In the subset of patients fulfilling the 20% response criteria of the American College of Rheumatology, a more pronounced reduction in the expression of ICAM-1, VCAM-1, IL-1β, and MMP-1 was found compared with the nonresponders.**

Conclusion. **Leflunomide and methotrexate are clinically efficacious drugs that interfere with mechanisms involved in joint inflammation and destruction of joint integrity.**

Rheumatoid arthritis (RA) is a chronic disease characterized by symmetric polyarticular inflammation, including the small joints of the hands, which is very frequently accompanied by destruction of joint integrity (1). Both inflammation and destruction lead to functional impairment and disability (2). The inflamed RA joint shows hyperplasia of the intimal lining layer and

Supported by a grant from Hoechst Marion Roussel.

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Submitted for publication December 17, 1999; accepted in revised form March 26, 2000.

increased cellularity of the synovial sublining. Predominant cell types involved in synovial inflammation include T cells (3), monocyte/macrophages (4), and fibroblast-like synoviocytes (5). Concurrent with the increased cellularity is an increased expression of adhesion molecules that are involved in cell trafficking (6,7) and of proinflammatory mediators such as cytokines (8). Previous studies have documented that macrophages, by virtue of their production of matrix metalloproteinases (MMPs), are important mediators of tissue destruction (9). Interstitial collagenase (MMP-1) plays a significant role in the matrix-degrading process observed in RA (9,10). The effects of MMP-1 are counterbalanced by inhibitors, such as tissue inhibitor of metalloproteinases 1 (TIMP-1) (11). Both MMP-1 and TIMP-1 are expressed in the rheumatoid synovium (9,12).

The so-called disease-modifying antirheumatic drugs (DMARDs) used in the last few decades were prescribed to control the inflammatory process of RA (13). Increasing information has demonstrated that DMARDs such as cyclosporin A (14), sulfasalazine (15,16), and methotrexate (17) reduce cartilage and bone destruction. Leflunomide is a recently developed DMARD that effectively reduces joint inflammation and its deleterious effects on joint integrity (16–18). Leflunomide is a prodrug that is rapidly converted in the cell into the active metabolite A77 1726. Leflunomide exerts its effects by various mechanisms; the proposed primary mode of action in RA is inhibition of the enzyme dihydroorotate dehydrogenase (19–21). Leflunomide also interferes with the phosphorylation of tyrosine kinases, resulting in effects on signal transduction pathways (22–24).

The aim of the present study was to provide insight into the mechanism of action of leflunomide on synovial tissue, the site of inflammation in RA.

PATIENTS AND METHODS

Patients. During a period of 14 months, 39 patients with active RA (25) were enrolled in a 2-center, prospective, randomized, double-blind, parallel-design clinical trial comparing leflunomide with methotrexate. Active disease was defined as ≥6 swollen or tender joints and levels of moderate or worse on the physician's and patient's assessments of disease activity. All patients had at least 1 clinically involved knee joint. Low-dose prednisone (<10 mg/day) and concomitant stable doses of nonsteroidal antiinflammatory drug (NSAID) treatment were allowed. None of the patients had ever taken leflunomide or methotrexate. In patients taking DMARDs, the treatment was stopped followed by a washout phase of 28 days.

Patients were randomized to receive either 20 mg/day of leflunomide, with a loading dose of 100 mg/day for the first 3 days, or 7.5 mg/week of methotrexate, increasing stepwise to 15.0 mg/week over 12 weeks. Clinical assessment at baseline and after 16 weeks included a 28-joint count for swelling and tenderness, physician's and patient's assessment of disease activity, duration of morning stiffness, patient's assessment of pain (by visual analog scale [VAS]), and levels of serum C-reactive protein (CRP). Clinical effects of the treatment regimens were calculated using the American College of Rheumatology (ACR) criteria for clinical response (26). All patients gave informed consent, and the study protocol was approved by the Medical Ethics committees of the Leiden University Medical Center and the Leeds University Hospital.

Arthroscopy. In all RA patients, arthroscopy was performed under local anesthesia at baseline and after 16 weeks of treatment (in the same knee). A small-bore 2.7-mm arthroscope (Storz, Tuttlingen, Germany) was used for imaging the synovium as well as for the biopsy procedure (27,28). At each arthroscopy, multiple synovial biopsy samples were taken throughout the entire joint, using a 2.0-mm forceps (Storz). If macroscopic variation of synovitis was present, samples were obtained from both macroscopically inflamed and macroscopically noninflamed regions (29,30). An average of 10 pieces of synovial tissue was used for immunohistology (31).

These specimens were directly collected en bloc in a mold embedded in TissueTek OCT (Miles Diagnostics, Elkhart, IN) and subsequently snap frozen by immersion in methylbutane (−80°C) after being randomly coded. The frozen blocks were stored in liquid nitrogen until they were processed. Shortly before staining, 5-μm sections were cut and mounted on glass slides (Star Frost adhesive slides, Knittelgläser, Germany); the slides were air dried at room temperature, carefully packed, sealed airtight, and stored at −80°C until immunohistochemical analysis could be performed.

Immunohistochemical analysis. Immunohistochemical staining was performed using a 3-step immunoperoxidase method, as described previously (32,33). Serial sections were stained with the following monoclonal antibodies: anti-CD3 (Leu-4 [T cells]; Becton Dickinson, San Jose, CA), anti-CD68 (EBM11 [macrophages]; Dako, Glostrup, Denmark), anti–intercellular adhesion molecule 1 (ICAM-1) (anti-CD54; MEM-11; Sanbio, Uden, The Netherlands), anti–vascular cell adhesion molecule 1 (VCAM-1) (anti-CD106; 1g11B1; Sanbio), anti–interleukin-1β (IL-1β) (LP-712; Genzyme, Cambridge, MA), anti–tumor necrosis factor α (TNFα) (IP-300; Genzyme), collagenase 1 (or MMP-1; R&D Systems Europe, Abingdon, UK), and TIMP-1 (Oncogene Research Products, Cambridge, MA).

Digital image analysis. Stained sections were analyzed for all markers using digital image analysis, as described previously (34). The digital image analysis system consists of several parts: a microscope with a motorized stage table (Zeiss, Germany), solid-state 3-chip charged coupled device (CCD) video camera (Sony, Tokyo, Japan), video digitizer (Matrox, Dorval, Quebec, Canada), and Qwin V2.2 image analysis software (Leica, Cambridge, UK) on a personal computer. For all markers, 1 representative region was used for image acquisition using 400× magnification, separating it into consecutive high-power fields (hpfs) with a 3-pixel overlap. Each hpf was digitized into a color image of 566 × 760 pixels, resulting in 430,160 pixels, each coded for 16,777,216 possible

Table 1. Clinical data on 16 RA patients treated with leflunomide and 19 RA patients treated with methotrexate, at baseline and after 4 months of treatment*

	Leflunomide			Methotrexate		
	Baseline	4 months	*P*	Baseline	4 months	*P*
Tender joint count	21 ± 1.7	10 ± 2.5	<0.005	19 ± 1.6	12 ± 2.1	0.002
Swollen joint count	16 ± 1.4	11 ± 1.6	0.02	17 ± 1.1	12 ± 1.8	<0.02
Physician's assessment of disease activity	3.6 ± 0.16	2.5 ± 0.18	0.002	3.6 ± 0.14	2.6 ± 0.21	0.002
Patient's assessment of disease activity	3.6 ± 0.13	2.7 ± 0.25	<0.01	3.6 ± 0.18	2.6 ± 0.19	0.001
Duration of morning stiffness	2.8 ± 1.47	0.6 ± 0.14	<0.02	1.4 ± 0.32	0.4 ± 0.12	<0.005
Pain (by VAS)	4.2 ± 0.53	2.7 ± 0.59	<0.02	3.7 ± 0.47	3.1 ± 0.47	NS
CRP level (mg/liter)	50 ± 14.1	29 ± 9.6	<0.05	46 ± 8.7	28 ± 8.5	0.005

* Values are the mean ± SEM. RA = rheumatoid arthritis; VAS = visual analog scale; NS = not significant; CRP = C-reactive protein.

color images (34) and stored as a JPEG file without compression on a writable CD-ROM. CD3, CD68, ICAM-1, VCAM-1, TNFα, and IL-1β were evaluated by analyzing 6 consecutive hpfs; MMP-1 and TIMP-1 expression were analyzed on 20 consecutive hpfs. The acquisition procedures for each marker were performed in a single session using a standardized protocol (34–36).

For the evaluation of the scanned hpfs, 2 specialized programs were written in QUIPS (Leica). To evaluate cellularity (defined as the number of nuclei present) and infiltration by CD68+ cells and CD3+ cells, the software algorithms quantified the number of positively stained cells (34). For CD68+ cells, we discriminated between expression of these markers in the intimal lining layer and the synovial sublining, respectively (29). To quantify all other markers, an algorithm allowed the image area to be expressed in pixel units, as well as with a measure of mean optical density of the color product. After measurement, the integrated optical density (IOD) was calculated as the product of the stained area and the staining intensity (34,35,37); the IOD was subsequently corrected for the percentage of actual tissue in the analyzed areas.

Statistical analysis. The Wilcoxon signed rank test was used to determine significant differences within each treatment group, and the Mann-Whitney test was used for differences between the groups. Since this study had an explorative design, we did not use the Bonferroni correction. The Kendall correlation coefficient was calculated to see whether changes in one variable correlated with changes in others.

RESULTS

Patients. Thirty-nine patients were included in the study; paired synovial biopsy samples were available for 35 of them. One methotrexate-treated patient died during the study due to a myocardial infarction after 4 weeks of treatment, and 1 leflunomide-treated patient refused the second arthroscopy. Baseline synovial tissue biopsy samples from 1 patient in the leflunomide group and 1 patient in the methotrexate group were not assessable. The demographic characteristics of the 2 groups of RA patients were as follows: in the leflunomide group, 9 men and 7 women with a mean age of 60 years (range 35–77 years), a mean disease duration of 38

Table 2. Analysis of synovial tissue from 16 RA patients treated with leflunomide and 19 RA patients treated with methotrexate, at baseline and after 4 months of treatment*

	Leflunomide			Methotrexate		
	Baseline	4 months	*P*	Baseline	4 Months	*P*
Cellularity	1,551 ± 104	1,200 ± 129	<0.05	1,325 ± 104	1,211 ± 110	NS
Macrophages						
Intimal lining	70 ± 12	46 ± 9	NS	92 ± 15	53 ± 7	<0.05
Sublining	674 ± 90	421 ± 104	<0.05	689 ± 86	502 ± 91	NS
T cells	91 ± 36	40 ± 16	NS	183 ± 83	62 ± 27	NS
ICAM-1	53,514 ± 8,628	28,710 ± 4,686	0.01	66,109 ± 8,097	34,509 ± 6,199	<0.01
VCAM-1	42,780 ± 5,799	28,200 ± 5,955	0.05	46,116 ± 6,126	30,741 ± 5,512	NS
TNFα	10,696 ± 1,431	9,638 ± 1,225	NS	13,069 ± 1,392	11,465 ± 1,743	NS
IL-1β	56,055 ± 6,265	51,536 ± 7,753	NS	65,776 ± 4,693	42,941 ± 5,589	<0.01
MMP-1	5,133 ± 3,041	615 ± 225	0.002	1,721 ± 477	772 ± 279	<0.01
TIMP-1	12,836 ± 4,006	3,802 ± 1,401	<0.01	12,760 ± 4,915	8,281 ± 2,333	NS

* For assessments of cellularity, CD68+ macrophages, and T cells, values are the total (mean ± SEM) numbers in 6 high-power fields. For assessments of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), matrix metalloproteinase 1 (MMP-1), and tissue inhibitor of metalloproteinases 1 (TIMP-1), values are the mean ± SEM integrated optical density. RA = rheumatoid arthritis; NS = not significant.

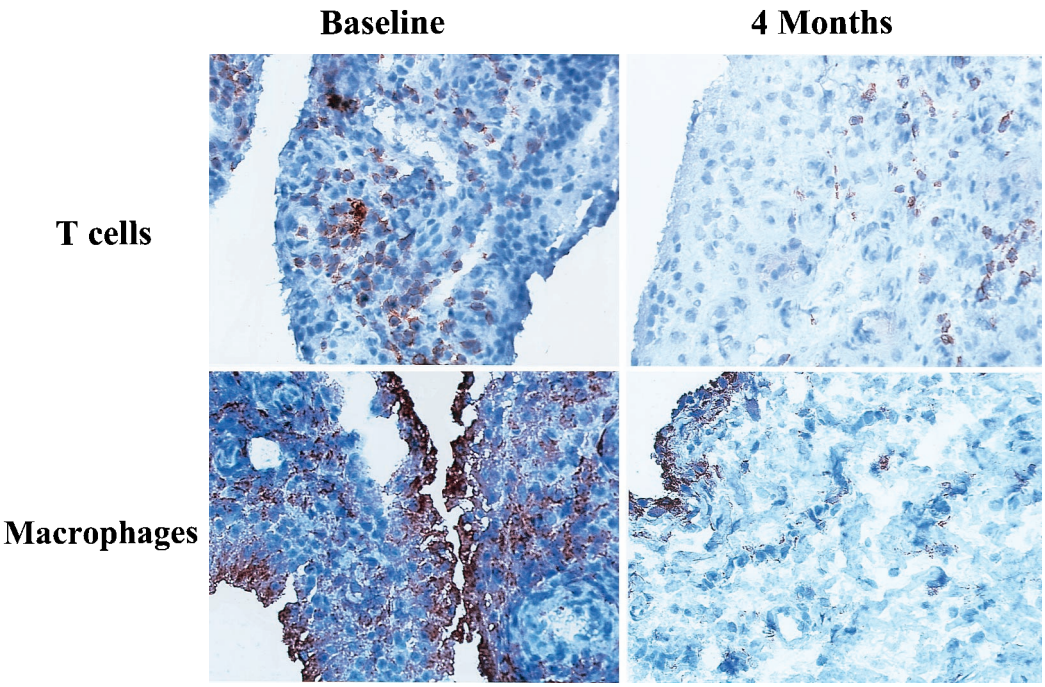

Figure 1. Rheumatoid synovial tissue before and 4 months after initiation of leflunomide therapy, showing a decrease in CD3+ T cells and CD68+ macrophages. Single-staining peroxidase technique; counterstained with Mayer's hemalum (original magnification × 400).

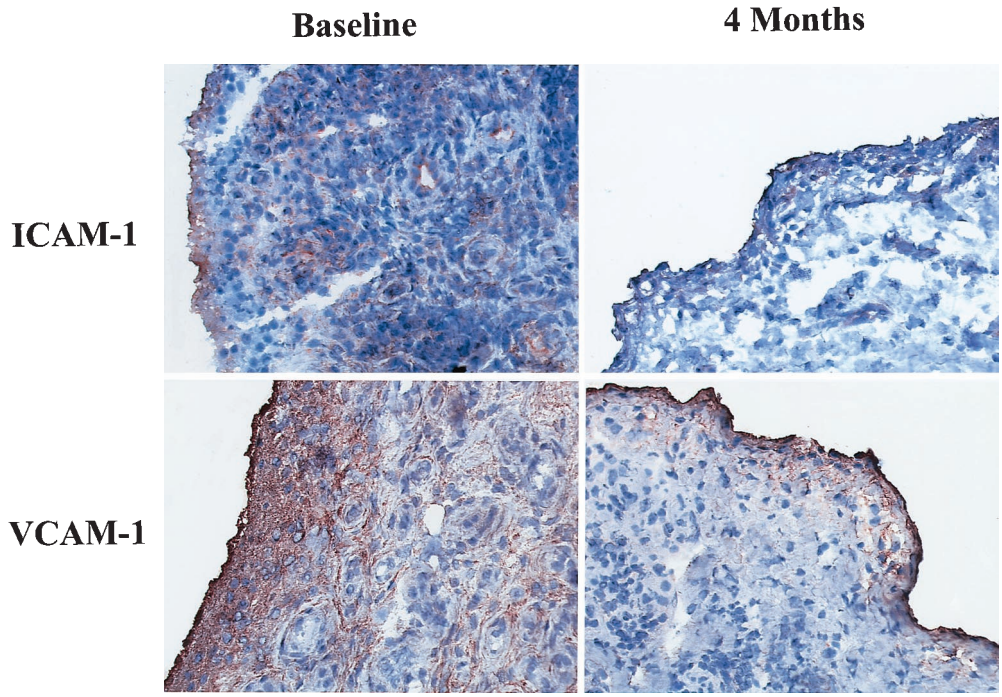

Figure 2. Rheumatoid synovial tissue before and 4 months after initiation of leflunomide therapy, showing a decrease in the expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). Single-staining peroxidase technique; counterstained with Mayer's hemalum (original magnification × 400).

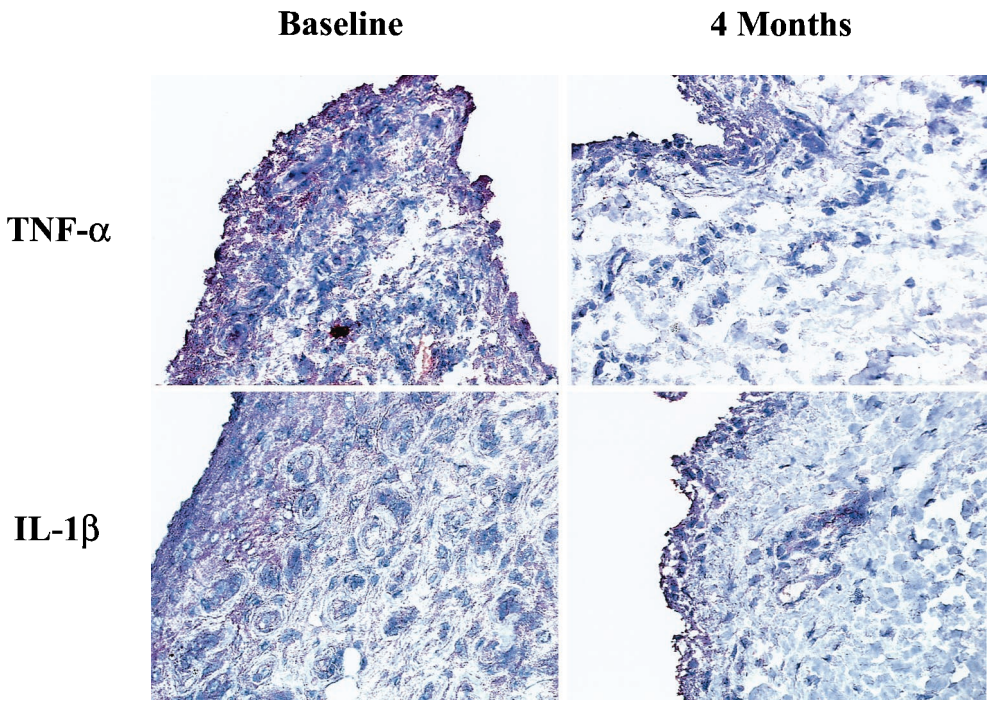

Figure 3. Rheumatoid synovial tissue before and 4 months after initiation of leflunomide therapy, showing a decrease in the expression of tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β). Single-staining peroxidase technique; counterstained with Mayer's hemalum (original magnification $\times$ 400).

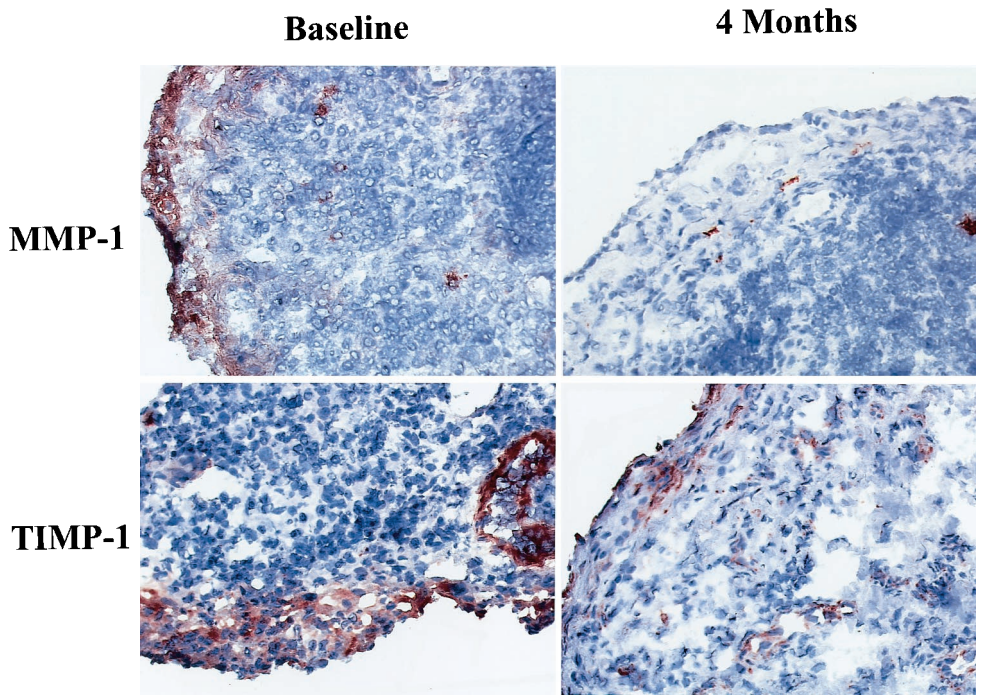

Figure 4. Rheumatoid synovial tissue before and 4 months after initiation of leflunomide therapy, showing a decrease in the expression of matrix metalloproteinase 1 (MMP-1) and tissue inhibitor of metalloproteinases 1 (TIMP-1). Single-staining peroxidase technique; counterstained with Mayer's hemalum (original magnification $\times$ 400).

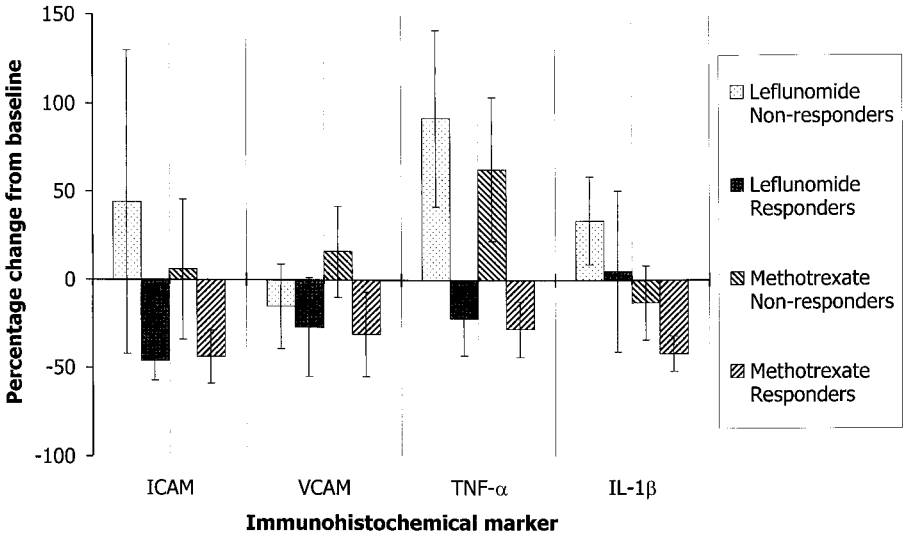

Figure 5. Mean ± SEM percentage change in the expression of intercellular adhesion molecule 1 (ICAM), vascular cell adhesion molecule 1 (VCAM), tumor necrosis factor α (TNF-α), and interleukin-1β (IL-1β) after 4 months of treatment with leflunomide or methotrexate. Responders fulfilled the American College of Rheumatology 20% response criteria.

months (range 2–138 months), and a mean of 1.1 (range 0–3) DMARDs previously taken; in the methotrexate group, 9 men and 10 women with a mean age of 59 years (range 27–77 years), a mean disease duration of 77 months (range 3–315 months), and a mean of 2.1 (range 0–4) DMARDs previously taken. One patient in the leflunomide group (6%) and 1 patient in the methotrexate group (5%) took 5 mg of prednisone daily.

Clinical efficacy. There was similar clinical efficacy of the instituted therapy in the 2 treatment groups, as demonstrated by changes in clinical parameters after 4 months compared with baseline (Table 1). Single exception was the VAS for pain, which was only significantly reduced in the leflunomide patients. Eight of the 16 patients treated with leflunomide (50%) and 10 of the 19 treated with methotrexate (53%) fulfilled the ACR 20% response criteria after 4 months of treatment. Four of the 16 leflunomide-treated patients (25%) and 4 of the 19 methotrexate-treated patients (21%) fulfilled the ACR 50% response criteria after 4 months of treatment.

Immunohistochemical analysis. The results of the immunohistochemical analysis at baseline and after 4 months are shown in Table 2. Baseline synovial tissue biopsy samples from 1 patient in the leflunomide group and 1 patient in the methotrexate group were not assessable; and these 2 patients were excluded from the immunohistochemical analysis.

Cellularity was especially reduced in the leflunomide-treated patients ($P < 0.05$) and remained relatively unchanged in the methotrexate-treated patients. Macrophage numbers were significantly reduced in the synovial sublining ($P < 0.05$) in the leflunomide-treated patients and in the intimal lining layer ($P < 0.05$) in the methotrexate-treated patients. T cell numbers were reduced in both groups, but the reduction did not reach statistical significance. ICAM-1 expression was significantly reduced in both the leflunomide ($P = 0.01$) and the methotrexate ($P < 0.01$) groups. VCAM-1 was reduced in both groups, but this difference was significant only in the leflunomide-treated patients ($P = 0.05$). TNFα was only slightly reduced in both groups. IL-1β was only moderately reduced in the leflunomide-treated patients; reductions in the methotrexate patients were significant ($P < 0.01$). MMP-1 was significantly reduced in both the leflunomide ($P = 0.002$) and the methotrexate ($P < 0.01$) groups. It should be noted that the baseline values for MMP-1 were 5,133 ± 3,041 in the leflunomide group compared with 1,721 ± 477 in the methotrexate group; this was due to 2 patients in the leflunomide group having high scores before the start of treatment. The level of TIMP-1 was significantly reduced in the leflunomide-treated patients ($P < 0.01$), but not the methotrexate-treated patients.

Representative examples of the immunohistochemical staining for CD3, CD68, ICAM-1, VCAM-1, TNFα, IL-1β, MMP-1, and TIMP-1 at baseline and after

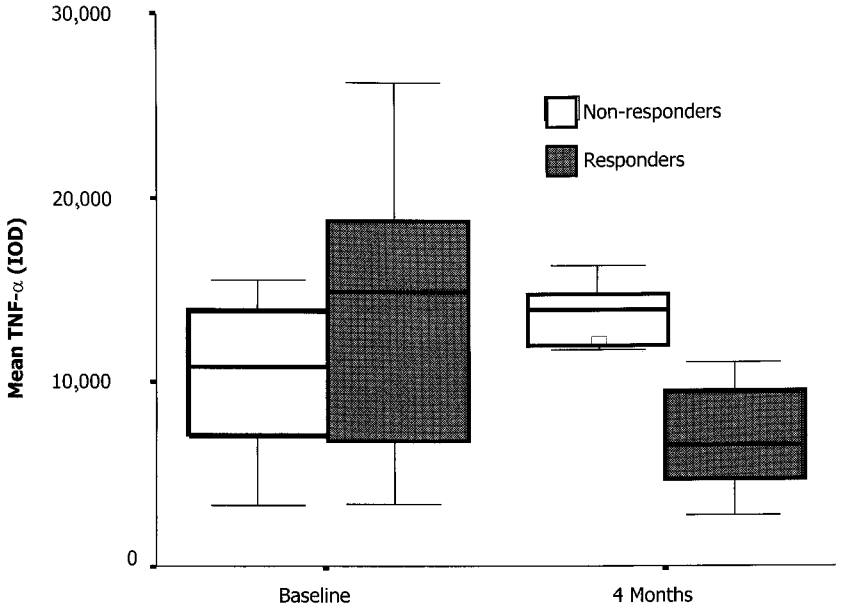

Figure 6. Mean ± SEM integrated optical density (IOD) values of tumor necrosis factor α (TNF-α) expression at baseline and after 4 months of treatment in relation to clinical response. Boxes show medians, quartiles, and extreme ranges.

4 months are shown in Figures 1–4. The mean ± SEM changes in the MMP-1:TIMP-1 ratio decreased more than 6-fold (6.4 ± 2.1) in the leflunomide group and ~5-fold (5.1 ± 2.0) in the methotrexate group. Differences in mean changes of immunohistologic scores between leflunomide- and methotrexate-treated patients were not statistically significant.

Immunohistochemical analysis in relation to clinical response. The mean ± SEM percentage change from baseline after 4 months of treatment for the markers ICAM-1, VCAM-1, TNFα, and IL-1β, are shown in Figure 5. The results are shown separately for the responders (fulfilling the ACR 20% response criteria) and the nonresponders.

In the leflunomide group, there was an increase in ICAM-1, TNFα, and IL-1β expression in the nonresponders. Responders displayed a reduction in expression of all markers, with the exception of IL-1β, which remained unchanged. There was a decrease in VCAM-1 expression in the nonresponders and a more pronounced reduction in the responders. Nonresponders in the methotrexate group showed an increase in ICAM-1, VCAM-1, and TNFα expression, whereas IL-1β expression decreased slightly.

Responders to methotrexate displayed a reduction in the expression of all 4 markers. As shown in Figure 6, the calculated Kendall correlation coefficient revealed that changes in the expression of TNFα correlated significantly with the clinical response, as defined by the ACR 20% response criteria ($P < 0.05$). None of the other immunohistologic parameters showed a correlation with clinical response. Leflunomide- and methotrexate-treated patients who fulfilled the ACR 50% response criteria showed a more pronounced response with regard to TNFα and MMP-1 expression compared with patients who fulfilled the ACR 20% response criteria, whereas all other markers showed a similar response (data not shown).

As depicted in Figure 7, the mean ± SEM MMP-1:TIMP-1 ratio was reduced more than 8-fold (8.8 ± 3.9) in the responders in the leflunomide group and more than 5-fold (5.3 ± 3.2) in the methotrexate group. In the nonresponders, the mean MMP-1:TIMP-1 ratio was reduced by a factor of 4.1 ± 1.5 in the responders in the leflunomide group and 4.7 ± 2.1 in the methotrexate group. None of the differences in mean changes between leflunomide and methotrexate were statistically significant.

DISCUSSION

In this study, we demonstrated that both leflunomide and methotrexate interfere with cellular infiltration. After 4 months of treatment, reduced numbers of

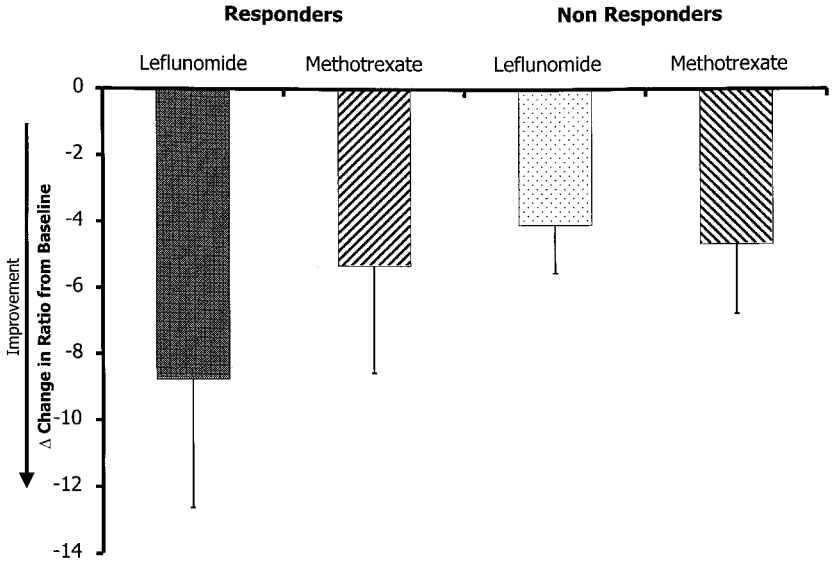

Figure 7. Mean and SEM change in the Δ matrix metalloproteinase 1 (MMP-1) to tissue inhibitor of metalloproteinases 1 (TIMP-1) ratio after 4 months of treatment in relation to the clinical response.

macrophages, and to a lesser extent T cells, infiltrated the synovium. This cellular reduction in infiltrates was found in conjunction with reduced expression of the adhesion molecules ICAM-1 and VCAM-1 and the proinflammatory cytokine TNFα in a similar manner for both treatments. With respect to mediators of tissue destruction, both leflunomide and methotrexate reduced the overall expression of MMP-1 and the MMP-1: TIMP-1 ratio after 4 months of treatment. The changes were more pronounced in patients who fulfilled the ACR 20% response criteria.

Synovial biopsies have been shown to be useful in the evaluation of RA treatment (38,39). Immunohistochemistry of synovial biopsy samples obtained in prospective studies with parallel design has been used to identify differences in the mode of action of various drugs. This appears to be especially beneficial for the evaluation of novel specific therapies such as TNFα blockade (40,41), and anti-CD4 monoclonal antibodies (32). For treatment with conventional DMARDs and biologics with diverse effects (42), the changes might be more diffuse and do not necessarily represent a specific effect (43–46). In this study, we observed, in general, a similar response after 4 months of treatment with either leflunomide or methotrexate. The data indicate that interference with pyrimidine biosynthesis (leflunomide) and interference with purine biosynthesis (methotrexate) ultimately have comparable effects on synovial inflammation.

Leflunomide reduced the total cellularity in synovial tissue, whereas there was only a moderate decrease in total cellularity after treatment with methotrexate, as demonstrated previously (44). The specific effect of leflunomide and methotrexate on the number of macrophages and T cells in synovial tissue samples was more pronounced at 4 months, and there was also a marked effect of both compounds on the expression of adhesion molecules, a finding consistent with previous observations after methotrexate treatment (43). These changes were even more noticeable in the patients who fulfilled the ACR 20% response criteria. This observation confirms that changes in synovial inflammation and clinical disease activity are coupled processes (33). The presence of signs of inflammation in synovial tissue after 4 months is consistent with the fact that synovial inflammation is observed in clinically uninvolved joints (30,47).

The in vivo effects of methotrexate on mediators of joint destruction have been previously studied in a 4-month open-label trial (44). In our study population, we observed a similar reduction in MMP-1 with relatively unchanged levels of TIMP-1, resulting in a decreased ratio of protease to its inhibitor. In the leflunomide-treated patients, we observed an even more pronounced reduction in the MMP-1:TIMP-1 ratio. Of

importance, the changes in the MMP-1:TIMP-1 ratio were especially present in clinical responders. This finding is consistent with the observation that factors associated with the signs and symptoms of inflammation (33), such as the proinflammatory cytokines TNFα and IL-1β, are also involved in the regulation of degradative proteinases (48–51). Accordingly, we found increased levels of TNFα and IL-1β in nonresponders and significantly decreased levels of TNFα in responders. The observation that beneficial changes in the MMP-1:TIMP-1 ratio were also found in those patients who did not fulfill the ACR 20% response criteria illustrates that inflammation and destruction may, in part, be separate processes.

The exact mechanisms of action of leflunomide and methotrexate are not completely understood. It has been suggested that the effects of methotrexate on the production of tissue-degrading factors are related to adenosine receptor stimulation (52) rather than to the effects on folate metabolism (53). The primary mode of action of leflunomide is thought to be inhibition of pyrimidine biosynthesis (21,53–57), but other mechanisms are involved as well (22,24,58,59). Direct cell–cell contact between activated T cells and monocytes might be involved in MMP-1 induction (60). Recently, leflunomide was shown to decrease the capacity of T cells to activate monocytes during cell–cell contact (61). On contact with T lymphocytes, the ratios of IL-1β:IL-1 receptor antagonist and MMP-1:TIMP-1 in monocytes were reduced in the presence of leflunomide. Effects of leflunomide on signal transduction, such as inhibition of phosphorylation of tyrosine kinase, could also play a role (22,24,58). More downstream effects in the signaling cascade might involve inhibition of nuclear factor κB (59). This could explain, in part, the effect of leflunomide on the changes in the production of proinflammatory cytokines and MMPs (12,51).

In conclusion, leflunomide and methotrexate both reduce the expression of adhesion molecules and proinflammatory cytokines together with matrix-degrading factors, thus interfering with both inflammation and tissue destruction.

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