Reduced Chemokine and Matrix Metalloproteinase Expression in Patients with Rheumatoid Arthritis Achieving Remission

AMEL KATRIB, MALCOLM D. SMITH, MICHAEL J. AHERN, JOHN SLAVOTINEK, LEANNE STAFFORD, CAROLYN CUELLO, JAMES V. BERTOUCH, H. PATRICK McNEIL, and PETER P. YOUSSEF

ABSTRACT. ***Objective.*** To quantify the changes in synovial expression of mediators of macrophage chemotaxis, matrix degradation, and macrophage infiltration in the synovial membrane of patients with rheumatoid arthritis (RA) achieving American College of Rheumatology (ACR) defined remission and radiological arrest.

Methods. Knee synovial biopsies were taken from a selected group of 18 patients with RA before and after treatment and immunostained with antibodies specific for CD68; the chemokines macrophage inflammatory protein (MIP)-1α and monocyte chemoattractant protein (MCP)-1; matrix metalloproteinases (MMP-1 and 3) and their inhibitors, the tissue inhibitors of metalloproteinases (TIMP-1 and 2); as well as isotype-specific negative controls. Immunostaining was quantified using a computer assisted color video image analysis system. Radiographs were performed before and after treatment and the Larsen score determined. Patients were arbitrarily divided into 2 groups: the radiological arrest group (defined as change in Larsen score ≤ 5 from baseline) and radiological progressors (defined as change in Larsen score > 5). Patients were classified according to ACR response criteria.

Results. In the 8 patients who achieved ACR defined remission, there were tendencies toward reductions in the synovial lining layer (LL) expression of MIP-1α by 36% (p = 0.1) and MCP-1 by 48% (p = 0.1). Significant reductions occurred in the expression of MMP-1, by 53% in the LL (p = 0.008) and 59% in synovial sublining layer (SL) (p = 0.02) and MMP-3, by 76% in LL (p = 0.02), and 72% in SL (p = 0.008), but not in TIMP expression. In this group of patients there were reductions in MMP:TIMP ratios, in particular the MMP-1:TIMP-1 ratio in the LL (p = 0.05), MMP-3:TIMP-1 ratio in the LL (p = 0.05) and SL (p = 0.008), and MMP-3:TIMP-2 ratio in the LL (p = 0.04) and SL (p = 0.08). In this group of patients CD68+ macrophage infiltration was significantly reduced in the LL by 59% (p = 0.008) and in the SL by 52% (p = 0.008), which corresponded with the reductions in chemokine expression. In the remaining 10 patients who did not achieve full remission there were no significant changes in the variables studied. In the group achieving ACR 50% or 70% response there was a reduction in CD68 expression that approached significance (p = 0.06 in LL and SL), but there was no significant change in the other variables. There were no significant changes in the patients with an ACR 20% response. In the radiological arrest group (12 patients) there was a 41% reduction in LL expression of MIP-1α (p = 0.05) and MMP-1 (p = 0.06). Reductions in MMP:TIMP expression were also noted, in particular in MMP-1:TIMP-1 expression in the LL (p = 0.04) and MMP-3:TIMP-1 in the SL (p = 0.01). There were corresponding reductions in CD68 expression by 49% (p = 0.009) in LL and by 42% (p = 0.0005) in SL. In the radiological progressors (6 patients) there were no significant reductions in mediator expression.

Conclusion. In RA, ACR defined remission is associated with reductions in MMP-1 and 3 expression, with a corresponding reduction in macrophage infiltration and a tendency to reduction in MIP-1α expression. Radiological arrest is associated with reductions in MMP-1 expression, and significant reductions in macrophage infiltration, MIP-1 expression, and MMP:TIMP ratio. (J Rheumatol 2003;30:10–21)

Key Indexing Terms:
RHEUMATOID ARTHRITIS MATRIX METALLOPROTEINASES SYNOVIUM
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From the Inflammation Research Unit, School of Pathology, University of New South Wales; Rheumatology Unit, Prince of Wales Hospital, Sydney; Repatriation General Hospital, Adelaide; and the Institute of Rheumatology and Orthopaedics, Royal Prince Alfred Hospital, Sydney, Australia.

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A. Katrib, FRACP; P.P. Youssef, PhD, FRACP, Institute of Rheumatology and Orthopaedics; M.D. Smith, PhD, FRACP, Associate Professor; M.J. Ahern, MD, FRACP, Associate Professor; J. Slavotinek, MD, FRACR,

Repatriation General Hospital; L. Stafford, FRACP, MMedSci (Clin Epi); C. Cuello, BSc, Inflammation Research Unit, School of Pathology, University of New South Wales; J.V. Bertouch, MD, FRACP, Rheumatology Unit, Prince of Wales Hospital; H.P. McNeil, PhD, FRACP Associate Professor, Inflammation Research Unit, School of Pathology, University of New South Wales, Rheumatology Unit, Prince of Wales Hospital.

We dedicate this paper to the memory of our beloved friend and colleague Dr. Leanne Stafford.

Address reprint requests to Dr. P.P. Youssef, Royal Prince Alfred Hospital, Institute of Rheumatology and Orthopaedics, QEII Building, 59 Missenden Rd., Camperdown, NSW 2050, Australia. E-mail: P.youssef@unsw.edu.au

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Rheumatoid arthritis (RA) is characterized by chronic synovial inflammation and early onset of progressive cartilage and bone destruction resulting in significant joint dysfunction[1,2]. Currently, the aims of therapy are to induce clinical remission and prevent radiological progression.

It has been observed that synovial membrane macrophage numbers correlate with joint erosion, suggesting a crucial role for macrophages in RA[3,4]. Various proinflammatory mediators may be involved in recruitment and activation of macrophages, in particular, the ß chemokines[5]. Studies have identified 2 members of this family, monocyte chemoattractant protein-1α (MCP-1α or CCL 2) and macrophage inflammatory protein-1α (MIP-1α or CCL 3), as important ß chemokines responsible for mononuclear cell accumulation into RA synovium[6-8]. In RA, the levels of MCP-1 and MIP-1α are higher in the synovial compartment than in osteoarthritis (OA)[7,8]. Although produced by a range of cell types in culture, macrophages appear to be the major source of these chemokines[6-8]. In addition to chemotaxis, ß chemokines activate leukocytes to induce cell adhesion molecules[9].

Irreparable degradation of the extracellular matrix in RA is at least partly mediated by the matrix metalloproteinases (MMP)[10-14]. MMP are a family of proteolytic enzymes that can degrade all components of the extracellular matrix and are responsible for both normal connective tissue remodeling and pathological tissue destruction in inflammatory diseases such as RA[15]. MMP are classified into 4 groups according to their substrate specificities: the collagenases, gelatinases, stromelysins, and membrane-type MMP. The natural inhibitors of MMP, the tissue inhibitors of metalloproteinases (TIMP), are produced in the RA synovial membrane[12,15]. It has been suggested that cartilage loss and joint destruction in RA may be due to local imbalance between activated MMP and TIMP[15-17]. Although several MMP and TIMP have been described in RA serum, synovial fluid, and synovial tissue, the best studied are MMP-1 and MMP-3. These have been found in higher concentrations in patients with RA than in those with OA and healthy controls[18]. We hypothesized that remission in RA would be associated with a marked reduction in chemokine expression and thus macrophage recruitment. This would result in a reduction in MMP expression, leading to a reduction in radiological progression and joint damage.

We compared the expression of the chemokines MCP-1 and MIP-1α, MMP-1 and 3 and their inhibitors TIMP-1 and 2, and macrophage infiltration in patients with RA who achieved remission with those who did not. We also compared the expression of these mediators in patients who developed radiological progression with those in whom there was radiological arrest.

Table 1. Demographic details of patients.

Patient	Age, yrs	Sex	Duration of Disease, yrs	CRP, mg/l	Rheumatoid Factor, (IU/ml)	DMARD Therapy	Change in Larsen Score	Response to Treatment
1	61	F	0.1	84	< 20	Gold/MTX/Pred	+2	ACR 50%
2	66	F	3	142	350	Gold/MTX	+2	ACR 50%
3	75	F	9	51	213	CYC	+8	ACR 20%
4	76	F	0.06	32	22	Gold	+8	ACR 20%
5	68	M	0.75	68	401	Gold, MTX	+2	ACR 70%
6	86	M	0.5	76	< 20	MTX	+1	ACR 20%
7	75	M	18	56	20	Gold	+12	Remission
8	60	F	5	80	28	MTX	+3	Remission
9	70	M	0.08	116	404	Gold, MTX	+2	Remission
10	59	F	0.15	82	20	Gold	+1	Remission
11	56	F	14	167	400	Gold/MTX	0	Remission
12	76	M	0.5	120	43	Pred, SSZ	+2	Remission
13	74	F	0.5	6	143	Gold/MTX	–7	ACR 50%
14	77	M	0.25	62	351	Gold	0	Remission
15	75	F	0.25	47	165	Gold	0	Remission
16	77	M	15	16	504	Gold	+10	ACR 50%
17	59	M	10	110	499	Gold/SSZ	+13	ACR 20%
18	73	F	18	116	335	Gold	+7	ACR < 20%

MTX: methotrexate; gold: intramuscular sodium aurothiomalate; SSZ: sulfasalazine; CYC: cyclosporin; Pred: prednisolone.

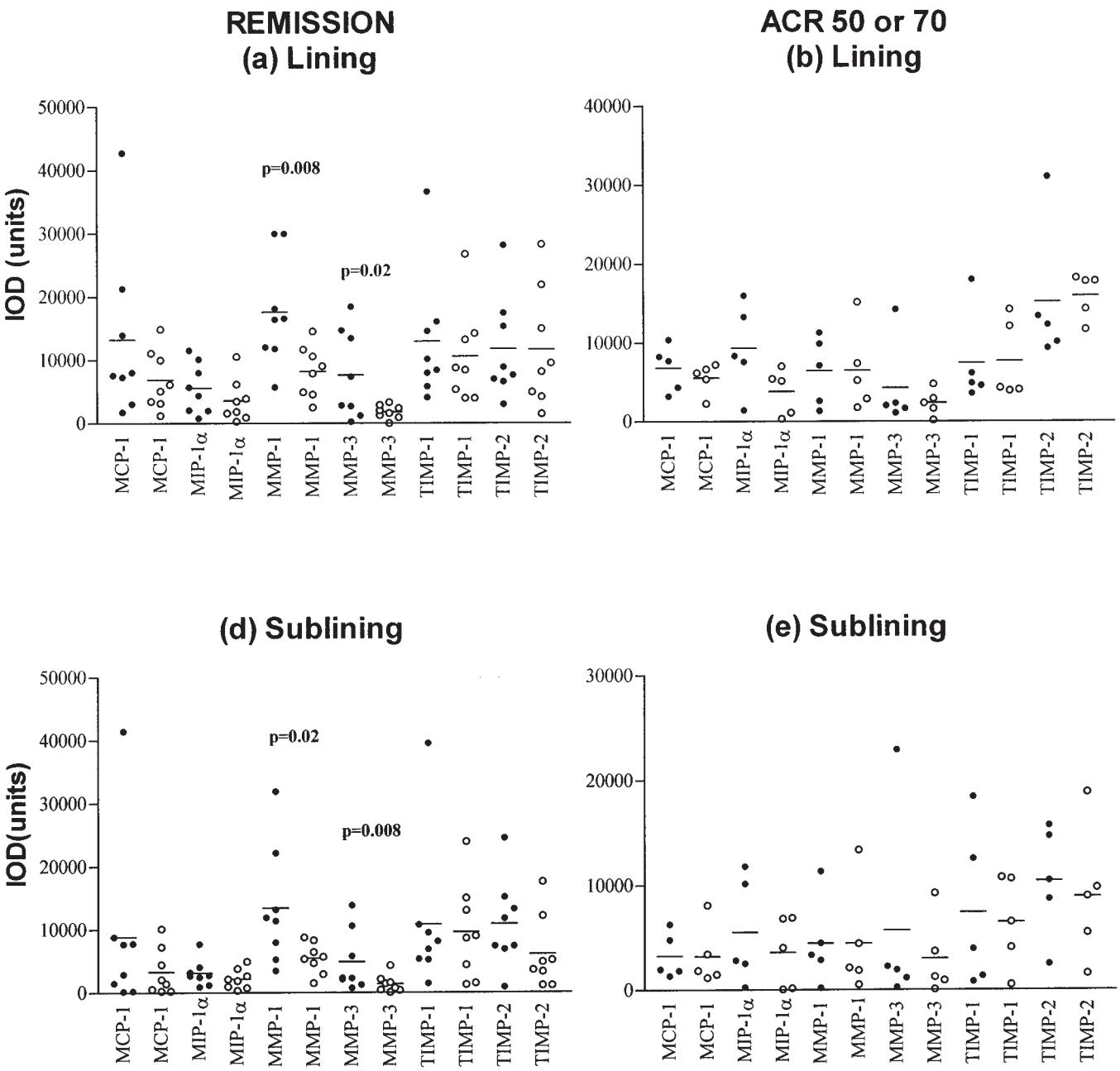


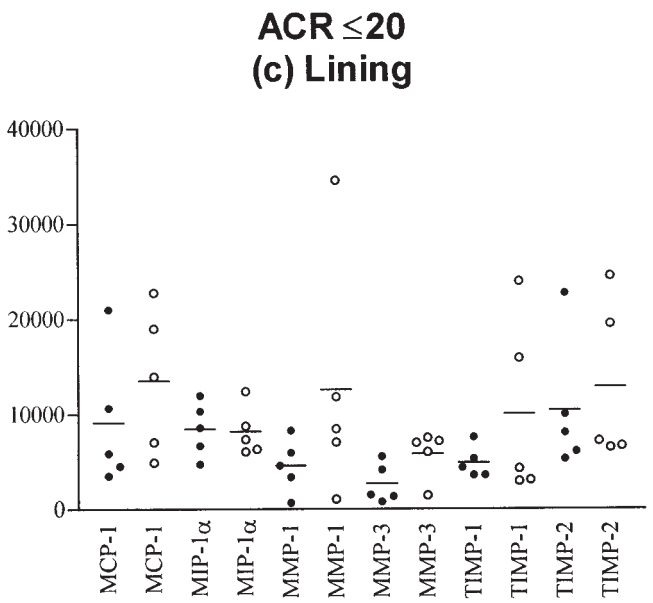
Figure 1. Effects of treatment on expression of chemokines, MMP, and their inhibitors (TIMP) in the synovial lining (A, B, C) and sublining (D, E, F) layers, in patients achieving ACR defined remission, or ACR 50%, 70%, or ≤ 20% remission. Significant reductions in the expression of MMP-1 and MMP-3 were observed in patients who achieved full ACR remission in both the lining and sublining layers. IOD: integrated optical density of staining. ●: pretreatment, ○: posttreatment.

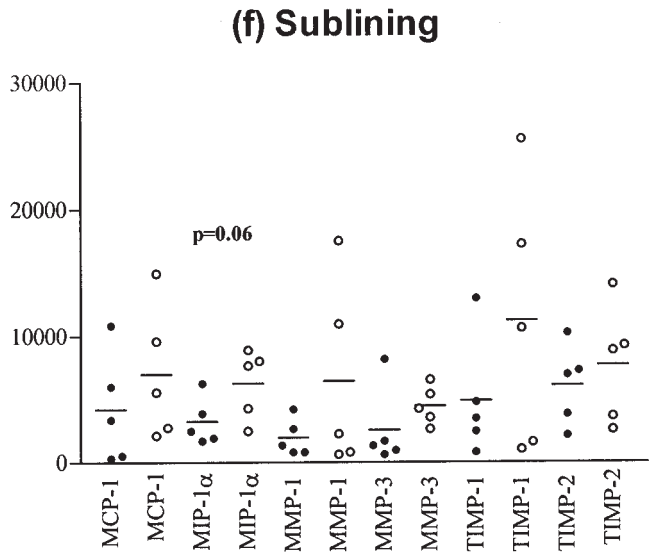
MATERIALS AND METHODS

Patients. The patients in this study are described in Table 1. All patients fulfilled the American College of Rheumatology (ACR) criteria for RA[19] and were selected from a larger cohort of RA patients being followed with sequential synovial biopsies after the initiation of disease modifying antirheumatic drug (DMARD) therapy. Of the 18 patients, 8 achieved ACR defined remission[20] after DMARD therapy and 10 failed to achieve remission. All patients gave their informed consent and the study protocol was approved by the Institutional Ethics Committee of the Repatriation General Hospital of Adelaide.

Clinical assessments were performed by a single observer on the day of synovial biopsy. The number of tender and swollen joints, patient and physician global assessments, and Health Assessment Questionnaire (HAQ) scores were obtained. Laboratory assessments included the determination of serum C-reactive protein (CRP) and rheumatoid factor (RF). Sequential radiological assessment of hands and feet was also performed and radiographs were scored using the Larsen index[21] by a single, independent observer (JS), who was blinded to clinical outcome.

Reagents. Sodium chloride and hydrogen peroxide were purchased from Merck (Victoria, Australia), methanol from Ajax Chemicals (Auburn, Australia), Tris base and diaminobenzidine (DAB) from Sigma (Castle Hill, Australia), bovine serum albumin (BSA) and proteinase K from





Boehringer Mannheim (Mannheim, Germany), normal goat serum, biotinylated goat anti-mouse secondary antibody and the avidin-biotin-horseradish peroxidase complex from Vector Australian Laboratory Services (Sydney, Australia), methyl green from BDH Chemicals (Sydney, Australia), and Eukitt from Lomb Scientific (Sydney, Australia).

Monoclonal antibodies (Mab). All antibodies used were murine Mab. Anti-MMP-1 (IgG2a), anti-MMP-3 (IgG1), anti-TIMP-1 antibodies (IgG1), and anti-TIMP-2 antibodies (IgG1) were purchased from ICN Biomedicals (Sydney, Australia). Anti-MIP-1 (IgG2a) and anti-MCP-1 (IgG2a) antibodies were purchased from R&D Systems Inc. (Minneapolis, MN, USA). Anti-CD68 Mab (PG-M1) (IgG3), irrelevant isotype-specific negative controls, and mouse IgG were purchased from Dako (Sydney, Australia).

Synovial tissue. Multiple biopsy specimens of synovial tissue were obtained arthroscopically from each patient as described[22]. Tissues were fixed in formalin and paraffin-embedded for immunohistologic analysis.

Immunoperoxidase staining. Serial sections were stained with the following Mab: anti-CD68, anti-MCP-1, anti-MIP-1α, anti-MMP-1, anti-MMP-3, anti-TIMP-1, anti-TIMP-2, and with irrelevant isotype-specific negative controls (Figure 3).

Formalin-fixed adjacent tissue sections (4 µm thickness) were digested and quenched for endogenous peroxidase staining as described[23]. For MIP-1α, MCP-1, and CD68 staining, slides were incubated in 25 µg/ml proteinase K for 20 min at 37°C. Slides were washed with Tris buffered saline (TBS), pH 7.6 (10 × TBS: 250 mM Tris base, 250 mM Tris HCl, 8.5% NaCl), then incubated with 20% goat serum for 20 min at room temperature to block nonspecific binding sites, followed by incubation with optimized dilutions of the primary antibody overnight in a humidified chamber at 4°C. Primary antibodies were diluted in 2% BSA/TBS. After further washes with TBS, the sections were incubated with biotinylated goat anti-mouse secondary antibody for 20 min at room temperature. The sections were washed again and an avidin-biotin-horseradish peroxidase complex was added for 60 min. After further washes, the sections were incubated with the DAB chromogen for 5 min and counterstained with methyl green, washed with 100% ethanol, and placed into xylene before being coverslipped with Eukitt. For each antibody, sections from all patients were processed in the same run. Negative controls were performed using irrelevant mouse isotype-specific controls, mouse IgG alone, or normal goat serum alone, or by omitting the secondary antibody. Specificity of staining was verified by adsorption of positive staining by preincubation with recombinant protein.

Color video image analysis. The immunostained sections were examined by computer assisted video analysis as described[22]. Measurements of the integrated optical density (IOD, a measure of the total amount of protein staining) and the mean optical density (MOD, equal to IOD divided by the area of DAB staining, which is a measure of the average concentration of protein on positively stained cells) were performed by a blinded observer (AK) who was unaware of the order of biopsies from any one patient or treatment outcome. The reproducibility of measurements was within 10% (data not shown). Differences were mostly due to variability in field selection. A minimum of 5 randomly selected high power fields were measured per synovial section.

Statistical analysis. Results are given as mean ± standard error of the mean (SEM). Comparisons were made using the Wilcoxon signed rank test. Differences were considered significant at $p \leq 0.05$. No adjustments were made for multiple comparisons.

RESULTS

The clinical features and treatment of the patient group are detailed in Table 1. Eight of the patients studied achieved full ACR remission. Ten patients did not achieve full ACR remission, of whom 5 achieved ACR 50% or 70% response and 5 achieved ≤ 20% response.

Remission versus nonremission. A comparison was made between patients who achieved ACR defined remission and those who did not. There were no significant differences between the remission group and the nonremission group with respect to age (mean ± SEM, 68.5 ± 1.5 vs 71.5 ± 2.6 yrs), sex (4 male, 4 female vs 4 male and 6 female), mean disease duration (4.8 ± 2.5 vs 5.7 ± 2.1 yrs), and mean Larsen score (26.25 ± 6.35 vs 36.2 ± 8.5) at study entry.

Remission is associated with reduction in MIP-1α expression. In the 8 patients achieving ACR defined remission, MIP-1α expression was reduced in the synovial lining layer

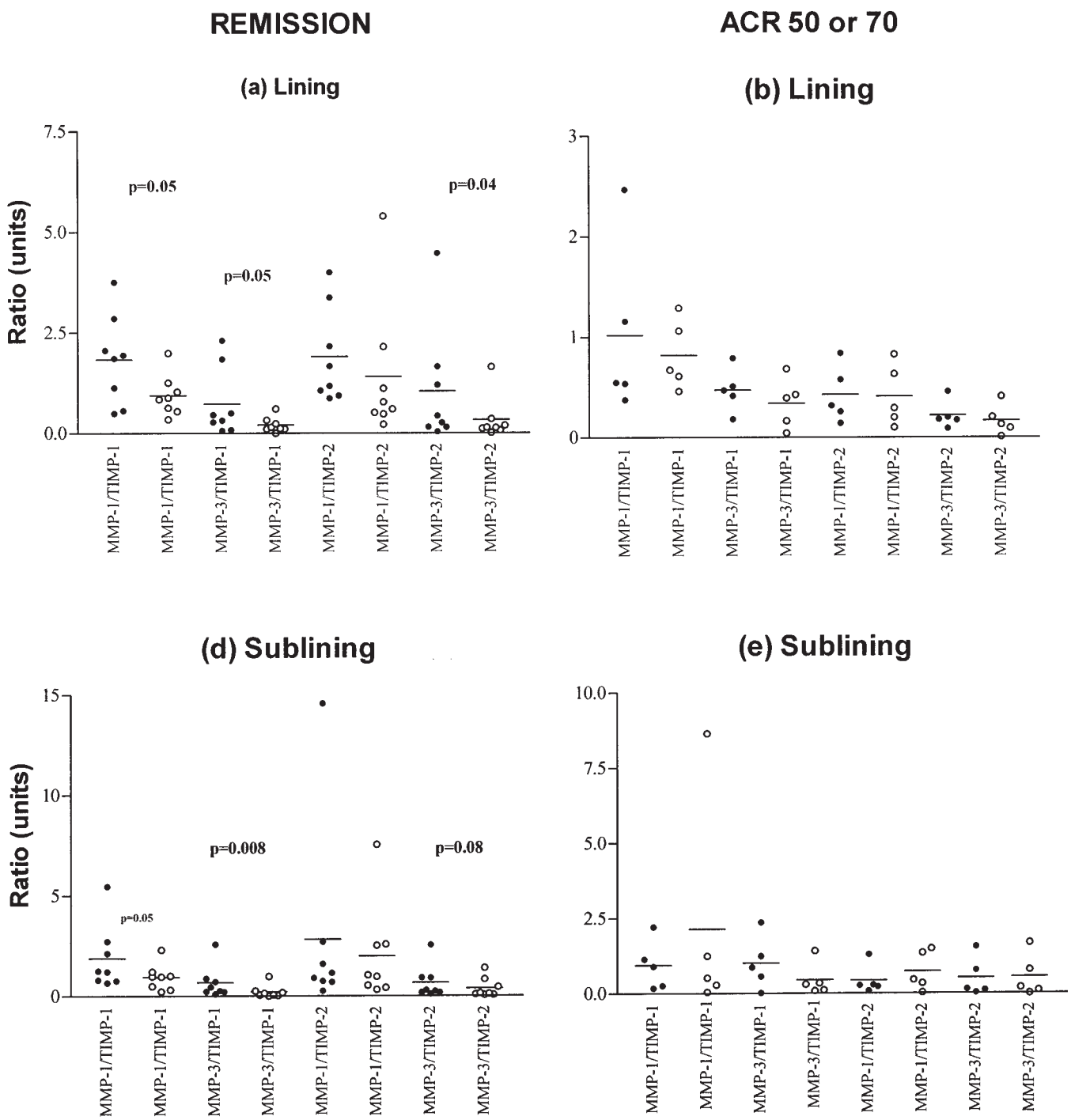
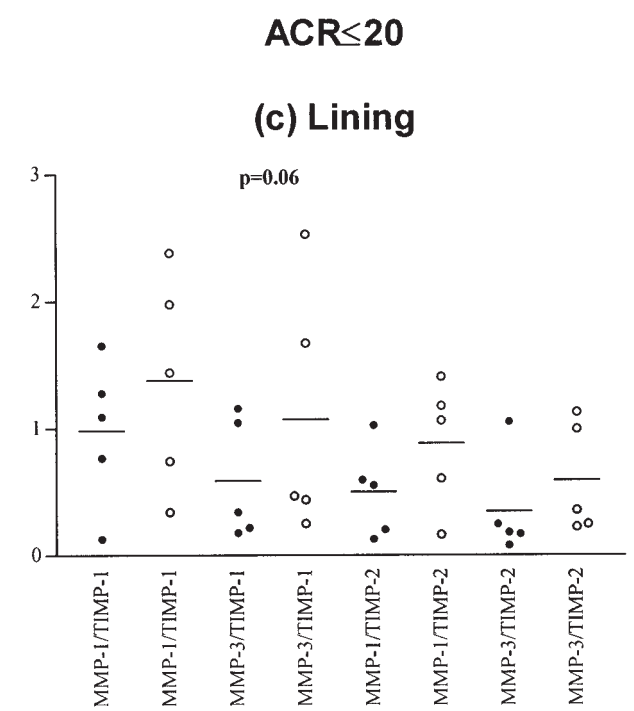
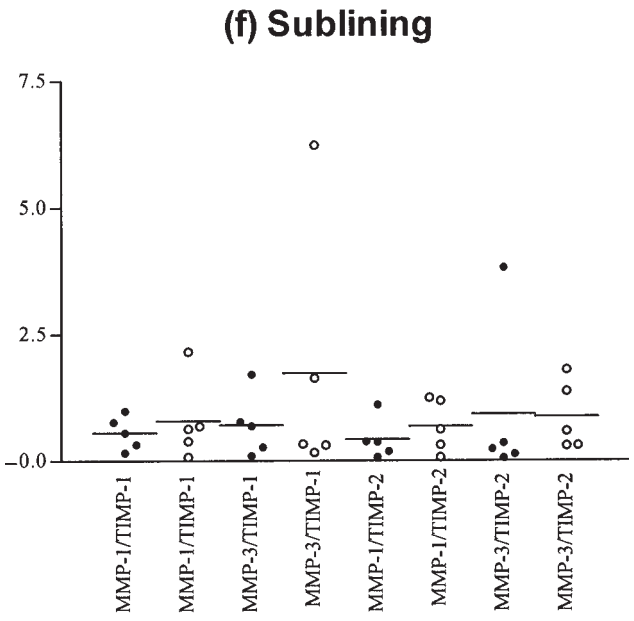


Figure 2. Effects of treatment on expression of MMP:TIMP in the synovial lining (A, B, C) and sublining (D, E, F) layers, in patients achieving ACR defined remission, or ACR 50%, 70%, or ≤ 20% remission. Significant reductions in the expression of MMP-1:TIMP-1, MMP-3:TIMP-1 were observed in lining and MMP-3:TIMP-1 in sublining layers in patients achieving full ACR remission. IOD: integrated optical density of staining. ●: pretreatment,○: posttreatment.

(LL) by a mean of 36% (p = 0.1) (Figures 3, 1A). There was a reduction of 48% in MCP-1 expression in the LL (p = 0.1) (Figure 1A) and 62% in the sublining layer (SL) (p = 0.3) (Figure 1D) that did not reach statistical significance.

Remission is associated with reduction in MMP-1 and MMP-3 but not TIMP expression. In patients who achieved ACR remission there were significant synovial LL reductions in MMP-1 and MMP-3 expression by 53% (p = 0.008) and by 76% (p = 0.02), respectively (Figures 3 and 1A), and in the SL by 59% (p = 0.02) and 72% (p = 0.008), respec-





tively (Figure 1D). There were no changes in TIMP-1 or TIMP-2 expression in either the LL (Figure 1A) or the SL (Figure 1D).

Remission is associated with reduction in MMP:TIMP ratio. In patients who achieved ACR remission there were reductions in MMP-1:TIMP-1 ratio by 49% (p = 0.05) in the LL (Figure 2A) and 50% (p = 0.1) in the SL (Figure 2D). MMP-3:TIMP-1 ratio was reduced by 71% (p = 0.05) in the LL (Figure 2A) and by 69% (p = 0.008) in the SL (Figure 2D). MMP-3:TIMP-2 was reduced by 68% (p = 0.04) in the LL (Figure 2A) and by 42% (p = 0.08) in SL (Figure 2D).

Remission is associated with reduction in macrophage infiltration. In patients achieving ACR remission, CD68+ macrophage infiltration was on average significantly decreased in the LL by 59% (p = 0.008) and in the SL by 52% (p = 0.008) (Figures 3 and 4A).

Nonremission group. Ten of the patients studied did not achieve full ACR remission. Of this group, 5 achieved ACR 50% or 70% remission and 5 achieved ACR ≤ 20% remission. Separate analysis of the 5 patients in the ACR 50% or 70% subgroup revealed a tendency toward reductions in MIP-1α and CD68 expression (Figures 1B, 1E, 4B), which were less than the reductions in the remission group and in the case of CD68 approached statistical significance (p = 0.06) in the LL and SL. There were no significant changes in the expression of the other mediators in the ACR 50% or 70% subgroup (Figures 1B, 1E).

Analysis of the subgroup achieving ACR ≤ 20% remission showed increased expression of MIP-1α in the SL that approached statistical significance (p = 0.06) (Figure 1F). Nonsignificant increases were observed in the expression of the remaining mediators (Figures 1C, 1F, 2C, 2F, 4C).

Mediator expression and radiological outcome. All patients had sequential radiographs of hands and feet at a mean ± SEM of 22.8 ± 3.5 months. Twelve patients were in the radiological arrest group (change in Larsen score ≤ 5) and 6 patients were in the radiological progressor group (change in Larsen score > 5).

Radiological arrest group. Of the 12 patients in the radiological arrest group, 7 achieved full ACR remission, 4 achieved ACR 50% or 70%, and one achieved ACR 20% remission. In this group, MIP-1α expression was significantly reduced in the LL by a mean of 41% (p = 0.05) (Figure 5A) but not in the SL (Figure 5C). There were reductions of 29% in MCP-1 expression in the LL (Figure 5A) and 39% in the SL (Figure 5C) that did not reach statistical significance.

There was a reduction in MMP-1 expression by 41% in the LL that approached significance (p = 0.06) (Figure 5A), and reductions in MMP-1 expression in the SL and MMP-3 expression in both the LL and SL that failed to reach statistical significance (Figures 5A, 5C). TIMP-1 and TIMP-2 expression was not significantly altered (Figures 5A, 5C).

In the radiological arrest group, there were significant reductions in MMP:TIMP expression, in particular in MMP-1:TIMP-1 expression by 43% in the LL (p = 0.04) (Figure 6A), MMP-3:TIMP-1 by 69% in the SL (p = 0.01) (Figure 6C), and MMP-3:TIMP-2 by 64% in the LL (p = 0.05) (Figure 6A). There were tendencies toward reductions in the

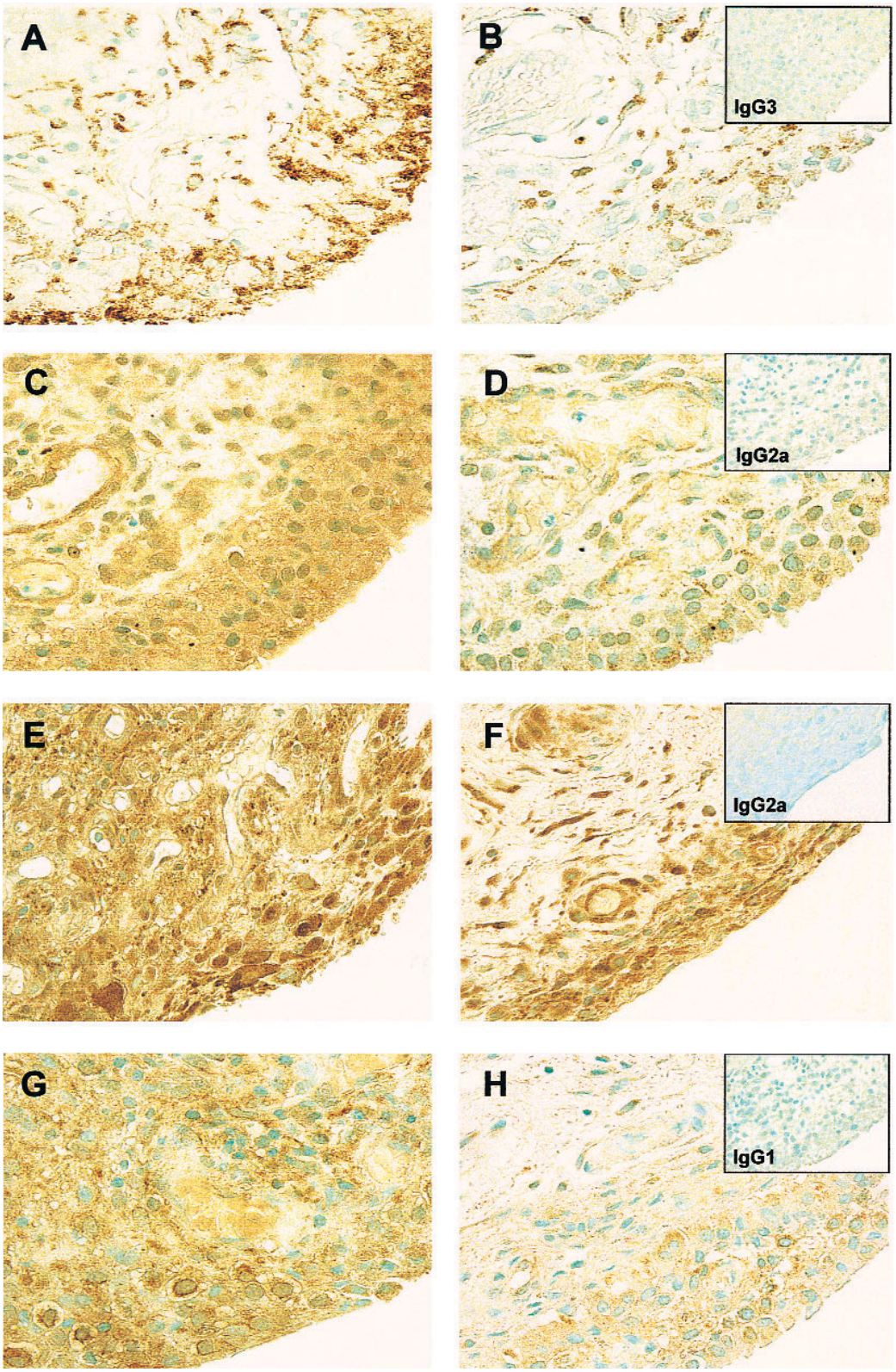


Figure 3. Representative photomicrographs (original magnification ×600) showing immunostaining patterns of knee synovial tissue taken before (panels A, C, E, G) and after achieving ACR defined remission (panels B, D, F, H). A and B, CD68 expression; C and D, MIP-1α expression; E and F, MMP-1 expression; G and H, MMP-3 expression. Negative controls shown in insets.

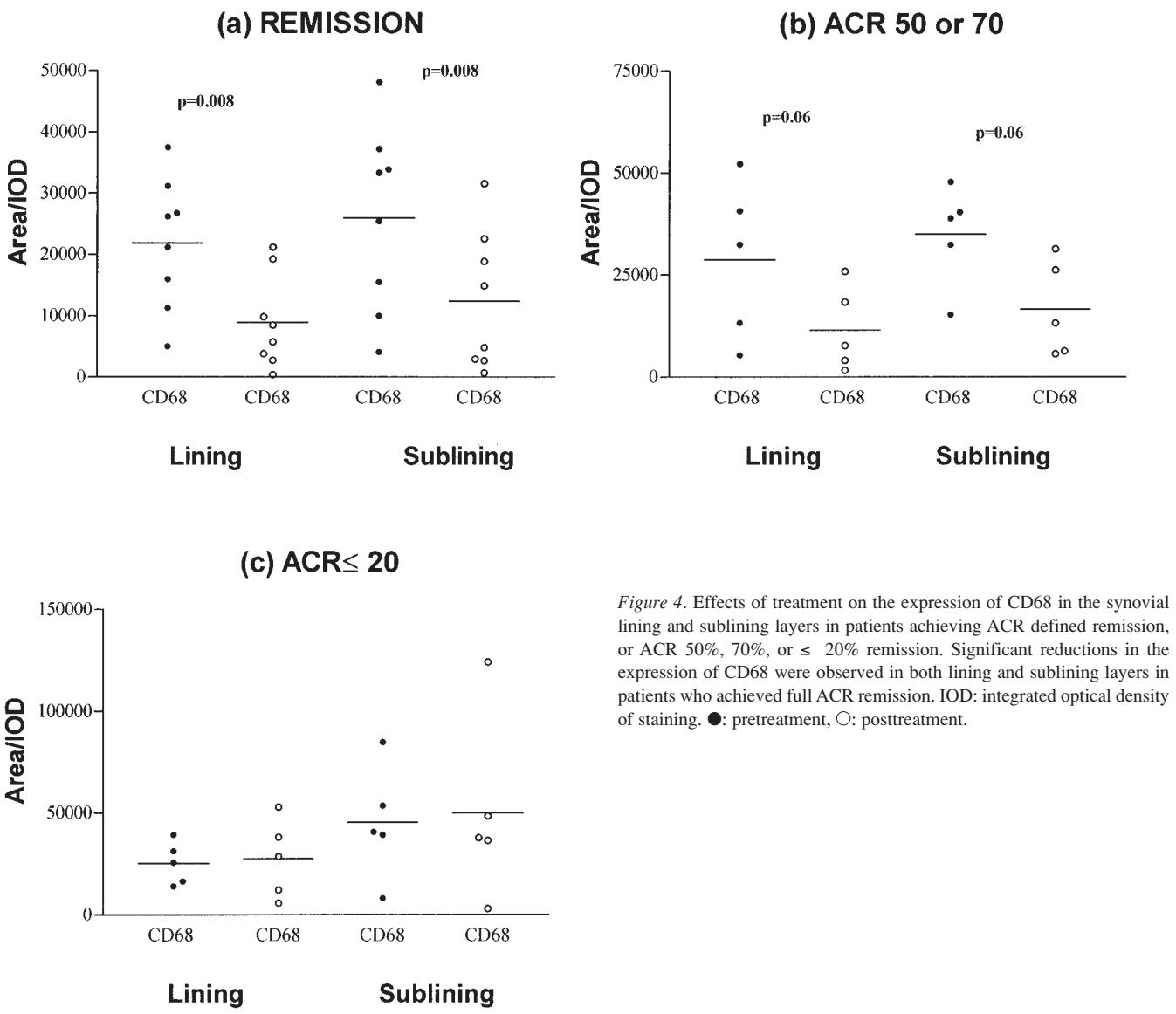


Figure 4. Effects of treatment on the expression of CD68 in the synovial lining and sublining layers in patients achieving ACR defined remission, or ACR 50%, 70%, or ≤ 20% remission. Significant reductions in the expression of CD68 were observed in both lining and sublining layers in patients who achieved full ACR remission. IOD: integrated optical density of staining. ●: pretreatment, ○: posttreatment.

remaining MMP:TIMP ratios that did not reach statistical significance (Figures 6A, 6C).

In the radiological arrest group CD68+ macrophage infiltration was on average significantly decreased in the LL by 49% (p = 0.009) and SL by 42% (p = 0.0005) (Figure 7A).

Radiological progressor group. Six patients showed radiological progression, of whom one achieved clinical remission, one achieved ACR 50%, and 4 achieved ACR ≤ 20% remission. There were no significant changes in chemokine, MMP, MMP:TIMP ratio, or CD68 expression in this subgroup (Figures 5B, 5D, 6B, 6D, 7B).

DISCUSSION

The balance between MMP and TIMP has been postulated to be relevant in the development of joint damage in RA[12,16,17]. A significant observation in this study was the reduction in MMP-1 and MMP-3 expression in both the lining and sublining layers in RA patients who achieved remission compared with those who did not achieve remission after DMARD therapy. There were also reductions in MMP:TIMP expression in the remission group of patients. Further, in patients in whom there was radiological arrest, there was a tendency toward reduction in MMP-1 and MMP-3, but no change in TIMP expression. Reductions were also observed in the MMP:TIMP expression in this group. In contrast, no significant reductions in MMP or MMP:TIMP expression were observed in patients in whom there was radiological progression. In a previous study, it was observed that MMP-1 expression occurs early in the synovial lining layer in RA and was associated with the presence of CD68+ macrophage infiltration and with new joint erosions[24]. That study and the current study support the

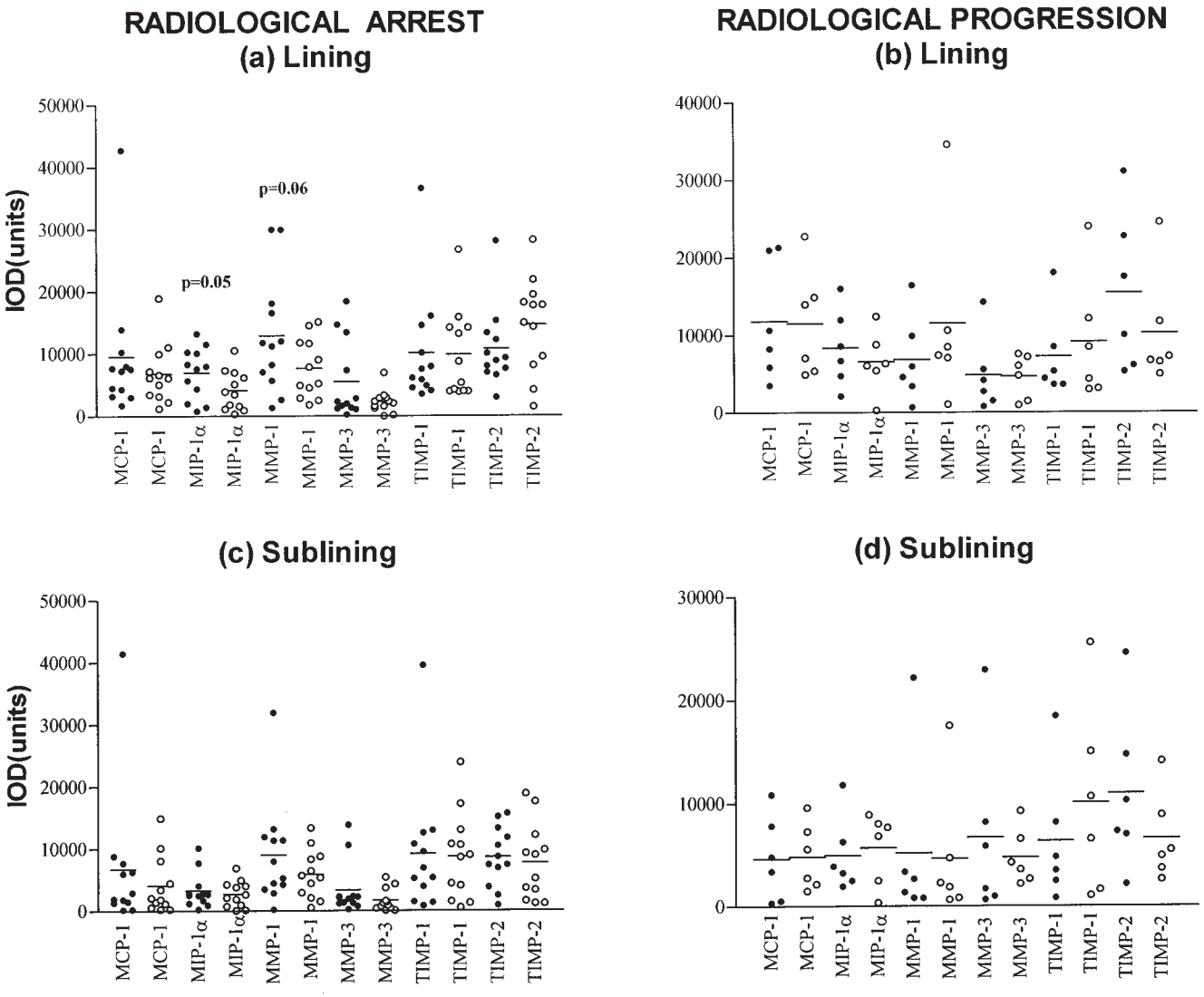


Figure 5. Expression of chemokines, MMP, and their inhibitors (TIMP) in the synovial lining (A and B) and sublining (C and D) layers in the radiological arrest group compared with radiological progressors (see Results section for definitions). Significant reductions in the expression of MIP-1α and almost significant reductions in MMP-1 in the lining layers were observed in the radiological arrest group. IOD: integrated optical density of staining. ●: pretreatment, ○: posttreatment.

development of specific MMP inhibitors for use in RA. Nonspecific MMP inhibitors have been reported to reduce cartilage breakdown in animal models of RA[25] and to be well tolerated in human studies in RA[26]. It remains to be determined whether these will alter disease progression.

Other major observations of this study were reductions of greater than 30% in the expression of MIP-1α and MCP-1 in patients achieving ACR defined clinical remission and in patients achieving radiological arrest. This is not surprising in view of findings in animal models, in which inhibition of MCP-1 and MIP-1α reduces the severity of arthritis, associated with a reduction in cellular infiltration of the synovial membrane[27,28]. Although there were similar qualitative changes in chemokine expression in patients achieving ACR 50% or ACR 70% responses, the reductions were less than in those patients achieving full ACR remission. In contrast, there was an increase in chemokine expression in the subgroup achieving only ACR ≤ 20% remission. We chose to study MIP-1α and MCP-1 because both human and animal studies have suggested a role for them in RA. These chemokines augment macrophage infiltration by their chemoattractant effects[5] and by activating cell adhesion molecules[9]. MIP-1α and MCP-1 are produced by macrophages, fibroblasts, and endothelial cells in the RA synovial membrane, in which their expression is increased compared with OA and normal synovium[6-8].

Significant reductions in macrophage infiltration in the synovial lining and sublining layers were observed in

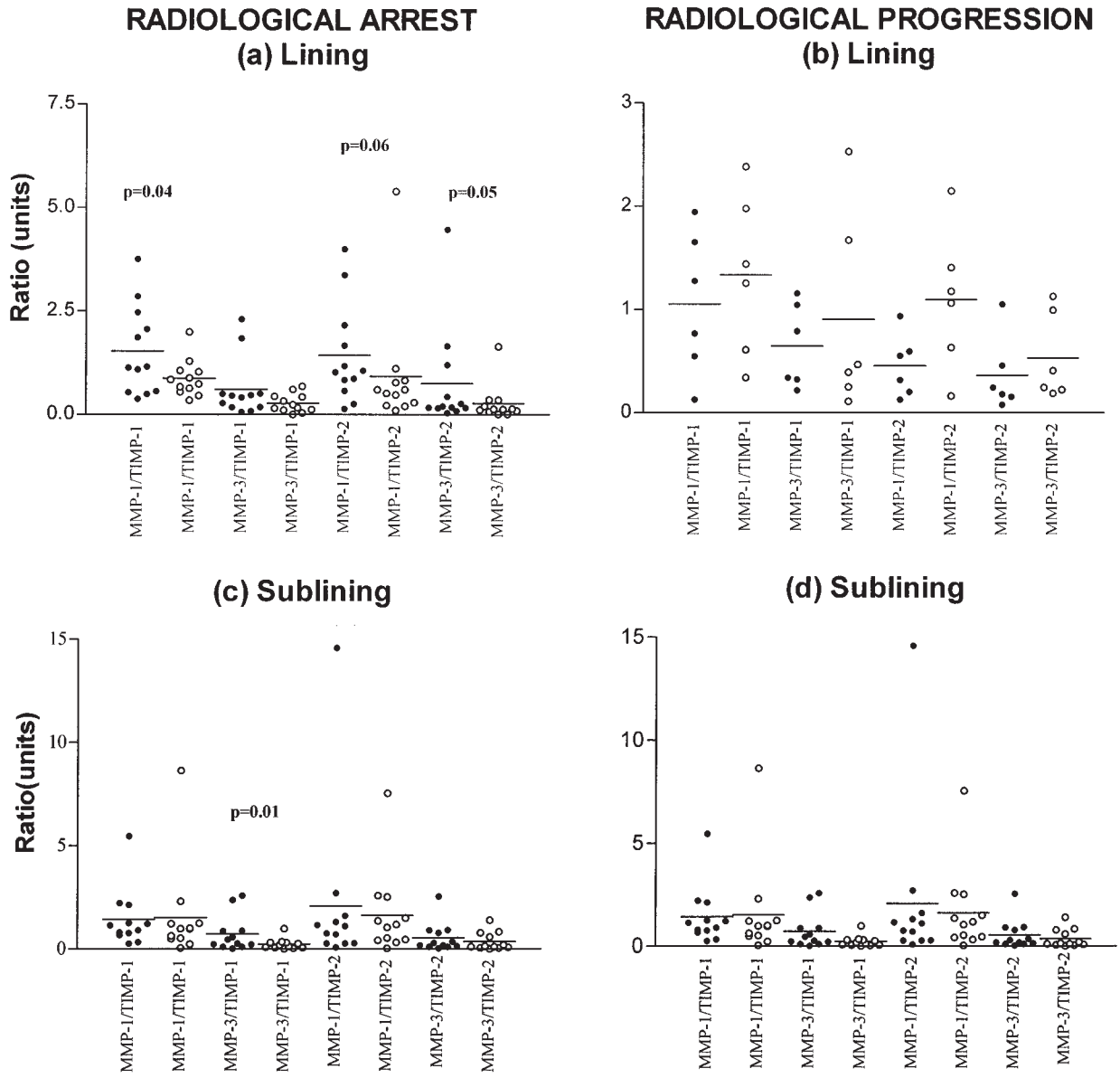


Figure 6. The expression of MMP:TIMP in the synovial lining (A and B) and sublining layers (C and D) in the radiological arrest group compared with radiological progressors (see Results section for definitions). Significant reductions in the expression of MMP-1:TIMP-1, MMP-3:TIMP-2 in the lining and MMP-3:TIMP-1 in the sublining layer, and almost significant reductions in the expression of MMP-1:TIMP-2 in the lining layers were observed in the radiological arrest group. IOD: integrated optical density of staining. ●: pretreatment, ○: posttreatment.

patients who achieved disease remission and in whom there was radiological arrest. This is not surprising, considering macrophages are important mediators of inflammation and joint damage in RA. Cross sectional studies have observed that macrophage numbers in the synovial lining predict radiological progression over 12 months[3,4]. Our study supports the role of synovial macrophages in joint destruction in RA and has the advantage of being longitudinal because synovial tissue was studied at 2 time points: before therapy, then after remission.

Our findings also suggest that the reductions in macrophage numbers in patients achieving ACR remission may be secondary to a reduction in the macrophage chemoattractants MIP-1α and MCP-1. To prove that reductions in chemokine expression were the direct cause of the reduction in macrophage numbers in humans, we would need to use specific inhibitors of chemokine function that are not yet available for clinical use. However, this study provides good circumstantial evidence for this assumption, and is supported by a study in which TNF inhibitors reduced MCP-1 expression and macrophage numbers in the RA synovial membrane[29]. Macrophages produce chemokines

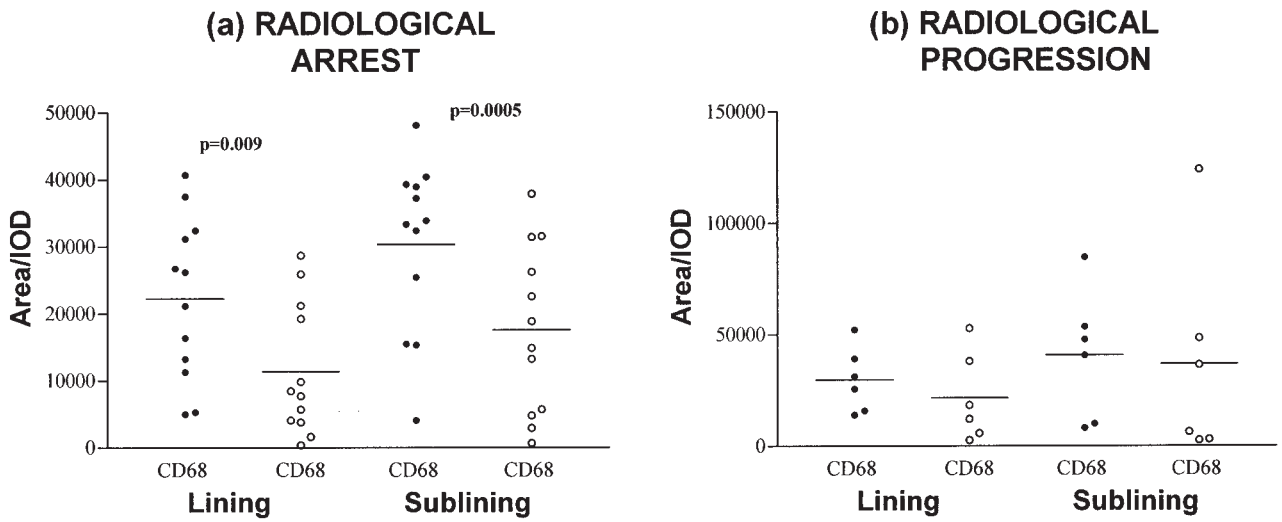


Figure 7. Expression of CD68 in the synovial lining and sublining layers in the radiological arrest group compared with radiological progressors (see Results section for definitions). CD68 expression was significantly reduced in both the lining and sublining layers in the radiological arrest group but not in radiological progressors. IOD: integrated optical density of staining. ●: pretreatment, ○: posttreatment.

and thus a reduction in chemokine expression may lead to a negative feedback loop in which there is a further reduction in macrophage numbers, followed by further reductions in chemokine expression and so forth. Reductions in synovial membrane macrophage numbers have been observed after methotrexate[30], gold[31], interleukin 1 receptor antagonist[32], and leflunomide therapy[33], and in the case of leflunomide, a concomitant reduction in chemokine expression was also observed.

This is a novel longitudinal study of chemokine, MMP, and TIMP expression as well as MMP:TIMP balance and macrophage infiltration in the rheumatoid synovial membrane. The observation that a reduction in macrophage numbers is associated with disease remission and radiological arrest supports the findings of cross sectional studies relating macrophage numbers to joint damage. Further, disease remission and radiological arrest were associated with some reductions in chemokine and MMP expression and reductions in MMP:TIMP ratio, confirming the results of *in vivo* and *in vitro* studies, suggesting a major role for these mediators and the possible importance of MMP:TIMP imbalance in RA and the therapeutic potential of specifically targeting these mediators.

REFERENCES

1. Brook A, Corbett M. Radiographic changes in early rheumatoid disease. Ann Rheum Dis 1977;36:71-3.
2. Pincus T, Callahan LF. Taking mortality in rheumatoid arthritis seriously — predictive markers, socioeconomic status and comorbidity. J Rheumatol 1986;13:841-5.
3. Mulherin D, FitzGerald O, Bresnihan B. Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. Arthritis Rheum 1996;39:115-24.
4. Yanni G, Whelan A, Feighery C, Bresnihan B. Synovial tissue macrophages and joint erosion in rheumatoid arthritis. Ann Rheum Dis 1994;53:39-44.
5. Luster AD. Chemokines — chemotactic cytokines that mediate inflammation. N Engl J Med 1998;338:436-45.
6. Akahoshi T, Wada C, Endo H, et al. Expression of monocyte chemotactic and activating factor in rheumatoid arthritis. Regulation of its production in synovial cells by interleukin-1 and tumor necrosis factor. Arthritis Rheum 1993;36:762-71.
7. Koch AE, Kunkel SL, Harlow LA, et al. Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. J Clin Invest 1992;90:772-9.
8. Koch AE, Kunkel SL, Harlow LA, et al. Macrophage inflammatory protein-1 alpha. A novel chemotactic cytokine for macrophages in rheumatoid arthritis. J Clin Invest 1994;93:921-8.
9. del Pozo MA, Sanchez Mateos P, Sanchez Madrid F. Cellular polarization induced by chemokines: a mechanism for leukocyte recruitment? Immunol Today 1996;17:127-31.
10. Tetlow LC, Woolley DE. Comparative immunolocalization studies of collagenase 1 and collagenase 3 production in the rheumatoid lesion, and by human chondrocytes and synoviocytes in vitro. Br J Rheumatol 1998;37:64-70.
11. McCachren SS, Haynes BF, Niedel JE. Localization of collagenase mRNA in rheumatoid arthritis synovium by in situ hybridization histochemistry. J Clin Immunol 1990;10:19-27.
12. McCachren SS. Expression of metalloproteinases and metalloproteinase inhibitor in human arthritic synovium. Arthritis Rheum 1991;34:1085-93.
13. Gravallese EM, Darling JM, Ladd AL, Katz JN, Glimcher LH. In situ hybridization studies of stromelysin and collagenase messenger RNA expression in rheumatoid synovium. Arthritis Rheum 1991;34:1076-84.
14. Keyszer G, Lambiri I, Nagel R, et al. Circulating levels of matrix metalloproteinases MMP-3 and MMP-1, tissue inhibitor of metalloproteinases 1 (TIMP-1), and MMP-1/TIMP-1 complex in rheumatic disease. Correlation with clinical activity of rheumatoid arthritis versus other surrogate markers. J Rheumatol 1999; 26:251-8.

15. Vincenti MP, Clark IM, Brinckerhoff CE. Using inhibitors of metalloproteinases to treat arthritis. Easier said than done? Arthritis Rheum 1994;37:1115-26.
16. Martel-Pelletier J, McCollum R, Fujimoto N, Obata K, Cloutier JM, Pelletier JP. Excess of metalloproteases over tissue inhibitor of metalloprotease may contribute to cartilage degradation in osteoarthritis and rheumatoid arthritis. Lab Invest 1994;70:807-15.
17. Firestein GS. Mechanisms of tissue destruction and cellular activation in rheumatoid arthritis. Curr Opin Rheumatol 1992;4:348-54.
18. Manicourt DH, Fujimoto N, Obata K, Thonar EJ. Levels of circulating collagenase, stromelysin-1, and tissue inhibitor of matrix metalloproteinases 1 in patients with rheumatoid arthritis. Relationship to serum levels of antigenic keratan sulfate and systemic parameters of inflammation. Arthritis Rheum 1995;38:1031-9.
19. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
20. Felson DT, Anderson JJ, Boers M, et al. American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995;38:727-35.
21. Larsen A, Thoen J. Hand radiography of 200 patients with rheumatoid arthritis repeated after an interval of one year. Scand J Rheumatol 1987;16:395-401.
22. Youssef PP, Triantafillou S, Parker A, et al. Effects of pulse methylprednisolone on cell adhesion molecules in the synovial membrane in rheumatoid arthritis. Reduced E-selectin and intercellular adhesion molecule 1 expression. Arthritis Rheum 1996;39:1970-9.
23. Di Girolamo N, Lloyd A, McCluskey P, Filipic M, Wakefield D. Increased expression of matrix metalloproteinases in vivo in scleritis tissue and in vitro in cultured human scleral fibroblasts. Am J Pathol 1997;150:653-66.
24. Cunnane G, FitzGerald O, Hummel KM, et al. Synovial tissue protease gene expression and joint erosions in early rheumatoid arthritis. Arthritis Rheum 2001;44:1744-53.
25. Lewis EJ, Bishop J, Bottomley KM, et al. Ro 32-3555, an orally active collagenase inhibitor, prevents cartilage breakdown in vitro and in vivo. Br J Pharmacol 1997;121:540-6.
26. Hemmings FJ, Farhan M, Rowland J, Banken L, Jain R. Tolerability and pharmacokinetics of the collagenase-selective inhibitor Trocade™ in patients with rheumatoid arthritis. Rheumatology 2001;40:537-43.
27. Kunkel SL, Lukacs N, Kasama T, Strieter RM. The role of chemokines in inflammatory joint disease. J Leukoc Biol 1996;59:6-12.
28. Gong JH, Ratkay LG, Waterfield JD, Clark Lewis I. An antagonist of monocyte chemoattractant protein 1 (MCP-1) inhibits arthritis in the MRL-lpr mouse model. J Exp Med 1997;186:131-7.
29. Taylor PC, Peters AM, Paleolog E, et al. Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor alpha blockade in patients with rheumatoid arthritis. Arthritis Rheum 2000;43:38-47.
30. Dolhain RJ, Tak PP, Dijkmans BA, De Kuiper P, Breedveld FC, Miltenburg AM. Methotrexate reduces inflammatory cell numbers, expression of monokines and of adhesion molecules in synovial tissue of patients with rheumatoid arthritis. Br J Rheumatol 1998;37:502-8.
31. Yanni G, Nabil M, Farahat MR, Poston RN, Panayi GS. Intramuscular gold decreases cytokine expression and macrophage numbers in the rheumatoid synovial membrane. Ann Rheum Dis 1994;53:315-22.
32. Cunnane G, Madigan A, Murphy E, FitzGerald O, Bresnihan B. The effects of treatment with interleukin-1 receptor antagonist on the inflamed synovial membrane in rheumatoid arthritis. Rheumatology 2001;40:62-9.
33. Kraan MC, de Koster BM, Elferink JG, Post WJ, Breedveld FC, Tak PP. Inhibition of neutrophil migration soon after initiation of treatment with leflunomide or methotrexate in patients with rheumatoid arthritis: findings in a prospective, randomized, double-blind clinical trial in fifteen patients. Arthritis Rheum 2000;43:1488-95.