Virchows Arch (2000) 436:449–458 

ORIGINAL ARTICLE

**A. König · V. Krenn · A. Toksoy · N. Gerhard**
**R. Gillitzer**

# Mig, GROα and RANTES messenger RNA expression in lining layer, infiltrates and different leucocyte populations of synovial tissue from patients with rheumatoid arthritis, psoriatic arthritis and osteoarthritis

Received: 19 August 1999 / Accepted: 17 January 2000

**Abstract** To investigate lymphocyte and monocyte recruitment-specific chemokine expression in synovial tissues from patients with rheumatoid arthritis (RA), psoriatic arthritis (PA) and osteoarthritis (OA), synovial membranes and cytocentrifuge preparations of 7 RA, 8 PA and 10 OA patients were examined by in situ hybridisation with antisense probes of Mig, GROα and RANTES and by immunohistochemistry. Patients' local disease activity (swelling and tenderness) in order to was graded and histological evaluation was performed compare these data with their chemokine expression profiles. Mig and RANTES hybridisation signals were detected in the synovial lining layer and in cellular infiltrates, whereas GROα expression was localised exclusively in the lining layer of PA and RA. Cytological analysis revealed Mig and GROα mRNA mainly in monocytic cells expressing KIM6, while RANTES mRNA was demonstrated predominantly in lymphocytic cells expressing CD3. In OA synovial membranes, significantly fewer hybridisation signals were present than in RA and PA synovial membranes. Patients with PA and RA had mild to severe local disease activity, whereas OA patients showed only mild disease activity. Histologically, PA and RA inflammatory scores ranged from 1 to 5, while OA synovium was consistently graded 1. Therefore, we conclude that the differential expression of Mig, GROα and RANTES in resident and in inflammatory cells has an important role in regulating leucocyte traffic in inflammatory arthropathies. The diverse leucocyte specificity of Mig, GROα and RANTES may thus regulate the recruitment of different leucocyte populations, as detected in PA and RA. Therefore, the pattern of cellular infiltration in human synovitis and the corresponding clinical signs of inflammation basically reflect the localisation and expression intensity of chemokines, which may be an important target for future disease modulation.

**Key words** Mig · GROα · RANTES · In situ hybridisation · Rheumatoid arthritis · Psoriatic arthritis · Osteoarthritis

**Abbreviations** *CC/CXC-chemokine* low-molecular-weight protein with the first two cysteines adjacent/separated by one amino acid · *ESR* erythrocyte sedimentation rate · *GROα* growth-related oncogene α · *IL-8* interleukin-8 · *IP 10* interferon-inducible 10-kDa protein · *MCP-1* monocyte chemoattractant protein-1 · *Mig* monokine induced by interferon gamma · *MIP* macrophage inflammatory protein · *MTX* methotrexate · *NSAID* nonsteroidal anti-inflammatory drug · *OA* osteoarthritis · *PA* psoriatic arthritis · *RA* rheumatoid arthritis · *RF* rheumatoid factor · *RANTES* regulated on activation · normal T cell expressed and secreted

A. König · N. Gerhard
Department of Orthopaedics, University of Würzburg, Brettreichstrasse 11, 97074 Würzburg, Germany

V. Krenn
Department of Pathology, University of Würzburg, Josef-Schneider-Strasse 2, 97080 Würzburg, Germany

A. Toksoy · R. Gillitzer
Department of Dermatology, University of Würzburg, Josef-Schneider-Strasse 2, 97080 Würzburg, Germany

A. König (✉)
Orthopädische Universitätsklinik, Brettreichstr. 11, 97074 Würzburg, Germany
e-mail: a-koenig.klh@mail.uni-wuerzburg.de
Tel.: +49-931-8031, Fax: +49-931-8031129

## Introduction

The role of various chemokines in regulating leucocyte accumulation in human tissues has been studied in several diseases, in particular of the skin [6, 8], and the results point toward a selective recruitment of distinct leucocyte populations induced by a differential chemokine expression in these conditions [6, 8]. However, there are only limited in vivo data on the role of chemokines in regulating the cellular infiltrate in inflammatory and noninflammatory arthropathies [15, 17, 28].

Rheumatoid arthritis (RA) is characterised histologically by an infiltration of mononuclear cells into the sy-

novial tissue, leading to enlargement of the synovial lining layer and the formation of cellular infiltrates and eventually follicles or granulomas [5]. Progression of the rheumatoid disease may ultimately lead to destruction of the joints and functional disability of the patient. Psoriatic arthritis (PA) is considered as a clinical entity distinct from RA [7, 23]. The synovial tissue in PA shows a mild inflammatory infiltrate consisting of lymphocytes and macrophages, with varying degrees of lining layer enlargement [17, 30]. In contrast, synovial tissues in osteoarthritis (OA) exhibit only minimal leucocyte infiltration and enlargement of the synovial intima. Hence, OA is classified as a noninflammatory arthropathy [5].

In the last decade a large number of highly homologous low-molecular-weight proteins have been discovered and subsumed under the CXC subfamily of chemokines. This family includes Mig, GROα, IL-8 and IP10. IL-8 and IP10 have been intensively studied both in vitro and in vivo, whereas data on GROα and, particularly, Mig are scarce. Mig seems to selectively activate and attract T lymphocytes [11, 12, 21], whereas GROα affects lymphocyte and neutrophil trafficking [9, 15]. RANTES, a member of the subfamily of CC-chemokines, has the capacity to act on distinct leucocyte populations, namely macrophages and memory T cells [29].

The selective chemoattractant activities of the CC- and CXC-chemokines for distinct leucocyte populations render them ideal candidates for a role in getting the appropriate leucocyte population to migrate into certain tissue compartments. Thus, the localisation and density of the different leucocyte populations may reflect the characteristic chemokine expression profile in inflammatory arthropathies.

The aim of the present study was to identify the sites of action of Mig, GROα and RANTES in the various regions of the synovium and to demonstrate the different leucocyte populations expressing these chemokines in inflammatory and noninflammatory arthropathies. We therefore used in vivo in situ hybridisation to examine the presence and distribution of Mig, GROα and RANTES mRNA in synovial membranes of patients with RA, PA and OA. The data on chemokine expression were compared with the density of the cellular infiltrate and enlargement of the synovial lining layer, and also with the disease activity in the joint. In addition, a comparative morphological approach was carried out using cytocentrifuge preparations of synovium, and the mRNA of Mig, GROα and RANTES was studied by in situ hybridisation in different mononuclear leucocytes identified both immunohistologically and morphologically.

## Patients and methods

### Patient population

The patients with RA ($n$=7), PA ($n$=8) and OA ($n$=10) underwent early synovectomy for active synovitis of joints and tendons or joint arthroplasty for arthritic joint destruction at the Department of Orthopaedic Surgery, König-Ludwig-Haus, University of Würzburg. Patients with PA met the criteria of Moll and Wright [23]. Patients with RA and idiopathic OA met the ACR criteria [1, 2]. The patients gave their informed consent, and the study was approved by the ethics review committee of the University of Würzburg. Resected synovial tissue from all patients was preserved for immediate preparation.

### Clinical assessment

The patients were all assessed by the same physician (A.K.), who evaluated the following parameters: number and localisation of affected joints and tendons and swelling and tenderness of the joint or tendon operated on. Swelling and tenderness were scored on a scale of 0–3 to obtain a grade of local disease activity of the joint operated on [13]: 0 for a normal joint, 1 for mild, 2 for moderate and 3 for severe abnormality. The ESR according to Westergren was determined from peripheral blood. Serum rheumatoid factors were analysed. Details of clinical characteristics are given in Tables 1–3. Radiological evaluation showed stage 4 or 5 disease [20] in the PA and RA patients with total joint replacements. All other PA and RA patients had stage 0–2. OA patients had radiological grade 3 or 4 disease [10].

### Tissue preparation

Part of each synovial membrane was immediately snap-frozen and part was immediately fixed in phosphate-buffered formalin and embedded in paraffin. Sections with microscopically visible inflammatory changes were chosen for further evaluation. In OA a representative section was used for evaluation. Another part of the tissue was used for cytological examination of synovial tissue cells in cytocentrifuge preparations. Tissue for cytological examination was prepared as described previously [18]. Immediately after synovectomy the tissue was minced and dissociated by treatment with 0.5 mg/ml of collagenase type 2 (Sigma, St. Louis, Mo.) in RPMI 1640 culture medium for 90–240 min at 37.5°C, followed by three filtrations through metal mesh (pore size 400 μm). The cells were resuspended in RPMI 1640/10% fetal calf serum culture medium and cytocentrifuge preparations were then carried out [18].

### Histological evaluation

The paraffin-embedded slides (Giemsa and haematoxylin/eosin staining) of tissue from all patients were used for standard histopathological evaluation and for scoring of the inflammatory infiltration on a semiquantitative scale of 1–5 [19]. As criteria the degree of enlargement of the synovial lining layer, the density and pattern of mononuclear infiltration and the extent of subsynovial fibrosis were used. All specimens were evaluated by two independent observers (A.K., V.K.), one of whom was unaware of the clinical diagnosis. Specimens were documented with an Axiophot microscope (Zeiss, Oberkochen, Germany).

### Cytological identification

In cytocentrifuge preparations five classes of cells were distinguished morphologically, as described previously [19]: multinucleated giant cells, monocytic cells with a large round to oval cytoplasm and a marginally located oval nucleus, lymphocytic cells with a small round cytoplasm and a large round nucleus, fibroblast-like cells with a dendritic or spindle shape and tube-like fragments of blood vessels. Cytological analysis was carried out by one observer, who was unaware of the clinical diagnosis (V.K.). In addition to the morphological identification immunohistochemistry was performed. Cytocentrifuge preparations of synovial membranes were used for the immunohistochemical detection of macrophages, T lymphocytes and vascular endothelium. Monoclonal antibodies (Dakopatts, Hamburg, Germany) directed against the antigens (specificities) CD3 (T

**Table 1** Characteristics of rheumatoid arthritis patients

| Patient | Age (years) | Sex | Tender-ness | Swelling | ESR | RF | Surgery | Disease duration | Medication | Inflammatory score (Histology) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 64 | f | 2 | 3 | 40 | + | Knee synovectomy | 10 years | NSAID | 4 |
| 2 | 59 | f | 1 | 2 | 17 | – | Metacarpophalangeal synovectomy | 3 years | NSAID, MTX, Steroid | 4 |
| 3 | 67 | f | 2 | 2 | 10 | + | Knee synovectomy | 3 years | NSAID, Steroid | 3 |
| 4 | 73 | f | 1 | 3 | 75 | + | Elbow synovectomy | 12 years | Sulphasalazin | 3 |
| 5 | 50 | f | 1 | 2 | 22 | + | Tenosynovectomy finger | 3 years | NSAID, Gold, Steroid | 5 |
| 6 | 64 | f | 2 | 2 | 14 | + | Knee synovectomy | 4 years | NSAID, Steroid | 3 |
| 7 | 64 | m | 2 | 2 | 36 | + | Tenosynovectomy wrist | 22 years | Sulphasalazin, Steroid | 3 |

**Table 2** Characteristics of psoriatic arthritis patients

| Patient | Age (years) | Sex | Tender-ness | Swelling | Clinical group | ESR | RF | Surgery | Disease duration | Medication | Inflammatory score (Histology) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 28 | m | 2 | 0 | Polyarticular | 55 | – | Hip arthroplasty | 13 years | NSAID | 4 |
| 2 | 29 | m | 0 | 2 | Oligoarticular | 15 | – | Knee synovectomy | 8 years | NSAID | 2 |
| 3 | 47 | f | 2 | 2 | Mutilans | 30 | – | Metacarpophalangeal synovectomy | 3 years | Steroid | 2 |
| 4 | 64 | f | 2 | 3 | Polyarticular | 32 | – | Wrist synovectomy | 13 years | NSAID | 3 |
| 5 | 61 | m | 2 | 3 | Oligoarticular | 24 | + | Knee arthroplasty | 1 year | – | 2 |
| 6 | 77 | f | 2 | 2 | Polyarticular | 75 | – | Knee arthroplasty | 8 years | MTX, Steroid | 4 |
| 7 | 36 | m | 1 | 2 | Oligoarticular | 14 | – | Knee synovectomy | 3 years | – | 1 |
| 8 | 32 | m | 2 | 2 | Oligoarticular | 44 | – | Tenosynovectomy finger | 2 years | NSAID, Steroid | 2 |

**Table 3** Characteristics of osteoarthritis patients

| Patient | Age (years) | Sex | Tender-ness | Swelling | ESR | Surgery | Inflammatory score (Histology) |
|---|---|---|---|---|---|---|---|
| 1 | 55 | m | 1 | 0 | 8 | Hip arthroplasty | 1 |
| 2 | 67 | f | 1 | 0 | 5 | Hip arthroplasty | 1 |
| 3 | 63 | m | 1 | 0 | 2 | Hip arthroplasty | 1 |
| 4 | 57 | f | 1 | 0 | 18 | Hip arthroplasty | 1 |
| 5 | 69 | f | 1 | 0 | 14 | Knee arthroplasty | 1 |
| 6 | 74 | f | 2 | 0 | 33 | Knee arthroplasty | 1 |
| 7 | 81 | f | 2 | 0 | 10 | Hip arthroplasty | 1 |
| 8 | 61 | f | 1 | 0 | 37 | Knee arthroplasty | 1 |
| 9 | 75 | f | 1 | 0 | 16 | Knee arthroplasty | 1 |
| 10 | 79 | f | 1 | 0 | 18 | Knee arthroplasty | 1 |

cells), KI-M6 (macrophages) and anti-factor-VIII-related antigen (vascular endothelium) were used in dilutions of 1:200 to 1:8000. As a secondary antibody a peroxidase-conjugated rabbit anti-mouse immunoglobulin diluted 1:10 in PBS supplemented with 2 vol% heat-inactivated normal human serum was used. Peroxidase activity was visualised with 0.06% diaminobenzidine (Walter, Kiel, Germany) and 0.01 vol% $H_2O_2$. No reliable specific antibody for fibroblasts is available. Therefore, the detection of fibroblasts was carried out on a morphological basis only. In all cases control stainings were obtained by replacing the primary antibody with PBS or a neutral antibody (pancytokeratin). Since the combination of radioactive in situ hybridisation and immunohistochemistry has not yet been demonstrated successfully for chemokines, morphological analysis and immunohistochemistry were performed to ensure a correct identification of the positive cells following in situ hybridisation. An analogous comparative cytological approach has been used before in rheumatoid synovial tissue [19].

## Preparation of RNA probes

cDNA probes were provided by: J.M. Farber (NIH, Bethesda, Md.), Mig; T. Schall (DNAX, Palo Alto, Calif.), RANTES; A. Anisowicz (Dana Farber Cancer Institute, Boston, Mass.), GROα. Subcloning of specific cDNA fragments in plasmids with SP6/T7 (pGEM, Promega, Madison, Wis.) or T3/T7 (Bluescript SK/KS, Stratagene, La Jolla, Calif.) initiation sites was done following standard protocols. In vitro transcription of sense and antisense probes was performed as described previously [6]. Radiolabeled probes were obtained by incubation of linearised plasmids with either T7, T3 or SP6 RNA polymerases (Boehringer Mannheim, Mannheim, Germany) and ATP, GTP, CTP (Boehringer) and $^{35}$S-UTP (Amersham, Braunschweig, Germany) as substrates. The original template cDNA was eliminated by DNase treatment, and protein components were removed by several phenol extraction steps. To facilitate the intracellular accessibility of labeled probes, alkaline hydrolysis was performed to give an average length of 50–150 bp. The position of the probes is in the coding region of the chemokine genes. The radioactive probes were adjusted to a specific activity of $2\times10^6$ cpm/µl in 0.01 M TRIS-HCI, pH 7.5, containing 1 mM EDTA.

## Hybridisation procedure

In situ hybridisation was performed as previously described [6, 8]. Cryostat sections were fixed for 20 min in 4% paraformaldehyde

in PBS and treated with proteinase K (1 µl/ml, Boehringer). Paraffin-embedded sections were deparaffinised in xylene and 100% alcohol and treated with 10 µg/ml proteinase K. After refixation in 4% paraformaldehyde, sections were acetylated with acetic anhydride in 0.1 M triethanolamine (pH 8.0, 10 min), dehydrated in alcohol and air-dried. Sections were then overlaid with 20 µl hybridisation solution (50% formamide, 300 mM NaCl, 20 mM TRIS-HCI, pH 8.0, 5 mM EDTA, 1× Denhardt's solution, 10% dextran sulphate, 100 mM dithiothreitol, and $2\times10^5$ cpm/µl heat-denaturated radioactive probe. The slides were hybridised at 46–47°C for 12–16 h. Every sense (control) and anti-sense probe was hybridised with three sections from the same biopsy. RNase treatment was used as a further control and consistently abrogated specific hybridisation signals. After hybridisation, sections were washed several times with a solution containing 50% formamide, 2× SSC (Sigma) and EDTA (5 mM) at high stringency (54–57°C). To remove unhybridised probes completely, slides were treated with RNase A (20 µl/ml) and RNase T1 (1 u/ml, Boehringer) for 30 min at 37°C. To visualise the hybridisation reaction, slides were dipped in NTB-2 solution (Kodak) and exposed for 1–4 weeks at 4°C. Developed slides of all specimens were evaluated by two independent observers (A.K., V.K.), one of whom was unaware of the clinical diagnosis. Specimens were documented with an Axiophot microscope.

### Evaluation of chemokine expression

In the sections of synovial membrane different regions were assessed separately: synovial lining layer, subsynovial cellular aggregates or infiltrates, and interstitial region. The number of cells expressing the chemokine in each region was estimated by counting the number of cells with hybridisation signals at three sites in the synovial membrane. Results were classified semiquantitatively on a 1–5 scale with minor modifications as described before [17, 18]: 1, 0–5%; 2, 6–15%; 3, 16–25%; 4, 26–35%; and 5, >35% of all cells positive. Chemokine expression in sections of synovial membrane from 6 patients with RA, 6 patients with PA and 10 patients with OA was assessed. The low availability of tissue with active synovitis limited the study of chemokine expression in sections of synovial membrane and cytological preparations of all patients.

In the cytological preparations 100 cells with positive signals were counted in three different regions of the cytological preparation and assigned into one of five classes. The percentage of positive cells per class was calculated. Mean percentage and range are given. Chemokine expression was assessed in cytological preparations from 5 patients with RA, 4 patients with PA and 1 patient with OA.

### Statistical analysis

The Mann-Whitney U-test was used to test whether there was a significant difference between RA, PA and OA for chemokine expression in sections of synovial membrane. Using the Bonferroni method to correct for multiple comparisons, only *P*-values of 0.002 or smaller were considered to be statistically significant. However, as this method is highly conservative, all *P*-values are shown. Owing to the limited number of cytological preparations no statistical testing was performed to compare the percentages of positive cells in the different classes.

## Results

Synovial membrane was obtained from 7 RA, 8 PA and 10 OA patients. RA and PA patients had active synovitis of joints or tendons with a mean disease duration of 8.1 (RA) and 6.4 years (PA). All PA patients had psoriasis vulgaris. Patients with PA and RA were mostly undergoing synovectomies, whereas in the patients with idiopathic OA only joint replacement surgery was performed. Patient age, sex, surgery, disease duration, clinical group of PA, ESR, rheumatoid factor and medication are detailed in Tables 1–3.

### In situ hybridisation of synovial membrane

In order to identify the microanatomical sites of expression, in situ hybridisation of synovial membranes was carried out. In a first series of experiments the expression of a battery of lymphocyte and monocyte-specific chemokines was studied. However, the expression of MIP1α, MIP1β and MCP was weak and inconsistent (data not given). Subsequently, analysis was restricted to Mig, GROα and RANTES. Hybridisation with Mig antisense probes, but not sense probes, revealed focally abundant silver grain precipitates in the synovial lining layer and in cellular infiltrates of psoriatic and rheumatoid synovium (Fig. 1A). Rheumatoid and psoriatic membranes with a less dense cellular infiltrate and thinner synovial lining layers seemed to exhibit a lower expression of Mig (Table 4). In contrast, osteoarthritic synovium showed a minimum of cell-associated hybridisation signals (Fig. 1B, Table 4).

Focal GROα expression was observed in the synovial lining cells of psoriatic and rheumatoid membranes (Fig. 2A). Only very few cell-associated GROα hybridisation signals could be detected in the cellular infiltrates of psoriatic and rheumatoid synovium (Table 4). This observation confirms the specificity of the hybridisation procedure, since no cross hybridisation occurred despite the high homology of Mig and GROα. Again, in osteoarthritic synovium only negligible amounts of GROα-mRNA could be detected (Table 4).

RANTES antisense probes showed positive cells in the synovial lining layer of psoriatic and rheumatoid synovium (Fig. 3A, Table 4). In cellular infiltrates prominent cell-associated labelling was detectable (Fig. 3A). Osteoarthritic synovium exhibited negligible cell-associated hybridisation signals (Table 4). Comparison of RA with PA synovial membrane revealed no significant difference in chemokine expression. When comparing OA with RA and PA significantly less chemokine is expressed in synovial lining layer and cellular infiltrates (Table 4).

### In situ hybridisation of cytocentrifuge preparations

Synovial tissue was dissociated, and hybridisation of these cytological preparations with sense and antisense probes of Mig, GROα and RANTES was performed. Cytological preparations of the same synovial membranes were examined by immunohistological staining for detection of macrophages, T lymphocytes and vascular endothelium to demonstrate the class of cells expressing the chemokine.

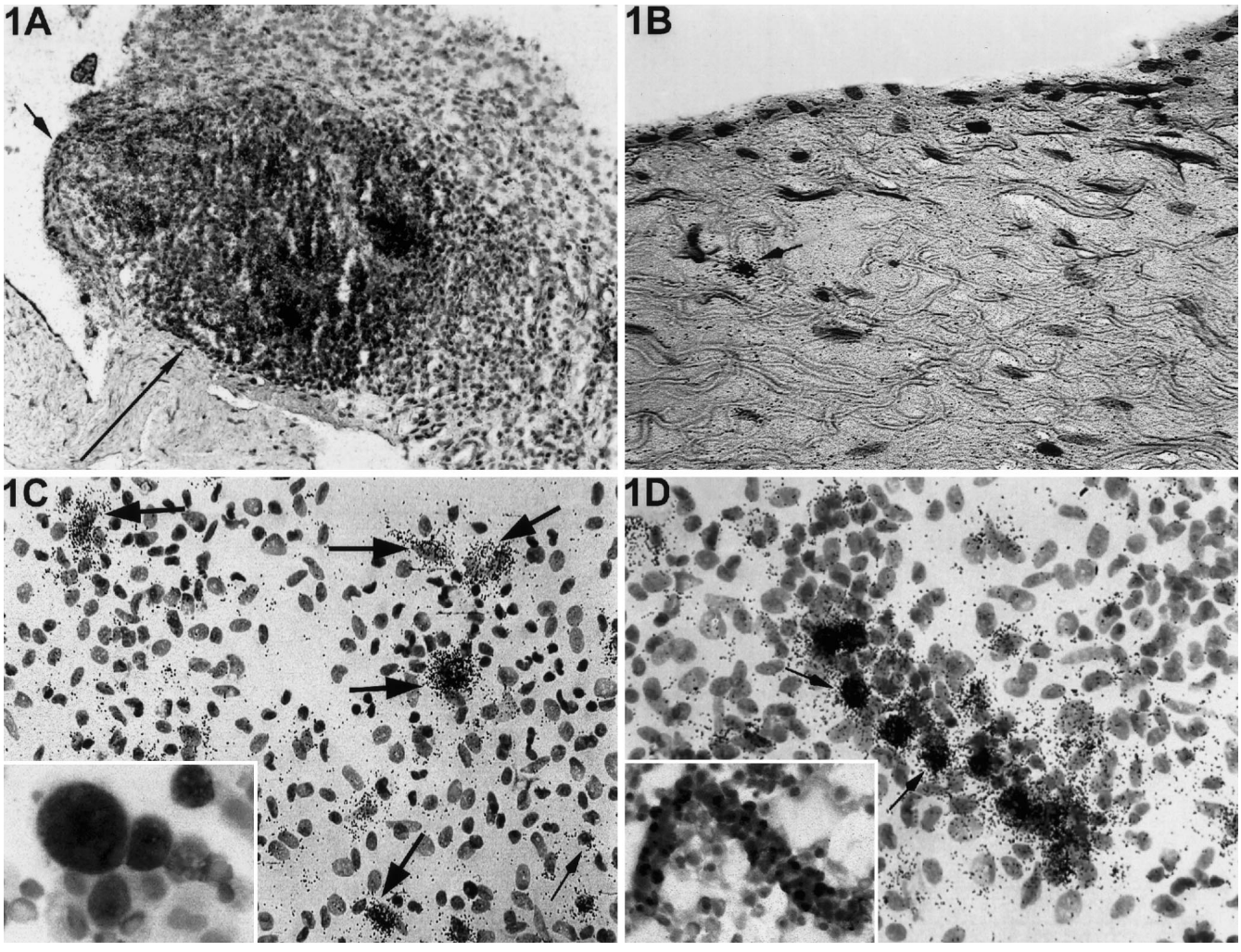


**Fig. 1A–D** Mig mRNA expression. **A** Section of synovial membrane from a rheumatoid arthritis patient showing Mig mRNA expression in the enlarged synovial lining layer (*small arrow*) and focal cellular infiltrates (*large arrow*). Original magnification ×150 **B** Section of synovial membrane from a patient with osteoarthritis exhibiting very low Mig mRNA expression in a lymphocytic cell (*small arrow*). Original magnification ×300 **C** Cytological preparation of rheumatoid synovium. Mig mRNA expression is localised mainly in monocytic cells (*thick arrows*) and only a few lymphocytic cells (*small arrow*) express Mig mRNA. Original magnification ×450 *Inset*: cytological preparation of synovium of the same patient showing monocytic cells expressing macrophage antigen Ki-M6. Original magnification ×1000 **D** Cytological preparation of rheumatoid synovium. Mig mRNA expression is seen in lymphocytic cells (small arrows) contained in the wall of a vessel fragment. Original magnification ×450 *Inset*: cytological preparation of synovium of the same patient showing a vessel fragment expressing anti-factor-VIII-related antigen. Original magnification ×300

In all preparations of psoriatic and rheumatoid synovium mainly monocytic cells demonstrated Mig (Fig 1 C, Table 5) hybridisation signals. These chemokine-expressing cells were KI-M6+ in immunohistological examination (Fig. 1C, inset). In addition, about 18% of the Mig mRNA+ cells had a lymphocytic morphology (Fig. 1D, Table 5). Notably, Mig hybridisation signals were detected in T lymphocytes situated in the vessel wall in some cytological preparations of psoriatic and rheumatoid synovium (Fig. 1D).

GROα hybridisation signals were detected mainly in monocytic cells in RA and PA (Fig. 2B, Table 5). No vessel-associated GROα signals were detectable. A lower percentage of cells had a lymphocytic morphology (Table 5).

RANTES mRNA expression was observed mainly in lymphocytic cells in RA and PA (Fig. 3B, Table 5). Immunohistology of the same cytological preparation revealed this cell type to be CD3+ (Fig. 3D). About one-fourth of the RANTES mRNA+ cells showed a monocytic appearance (Fig. 3C, Table 5).

Only very few positive chemokine signals could be detected in cell classes other than the monocytic and lymphocytic cell class in RA and PA (Table 5). In OA only negligible amounts of cell-associated chemokine signals could be found.

## Histological features

In order to compare the degree of synovial lining layer enlargement and the density of the cellular infiltrate with chemokine expression, histological grading of inflamma-

**Table 4** Expression of Mig, GROα and RANTES mRNA in various areas of synovial tissue from patients with rheumatoid arthritis (*RA*), psoriatic arthritis (*PA*) and osteoarthritis (*OA*). Expression was scored as follows: 1=0–5% positive cells; 2=6–15% positive cells; 3=16–25% positive cells; 4=26–35% positive cells; 5=>35% positive cells. (*NA* not available)

| Patient | Mig mRNA expression | | | GROα mRNA expression | | | RANTES mRNA expression | | |
|---|---|---|---|---|---|---|---|---|---|
| | Synovial lining layer | Cellular infiltrate | Interstitial region | Synovial lining layer | Cellular infiltrate | Interstitial region | Synovial lining layer | Cellular infiltrate | Interstitial region |
| RA 1 | NA | NA | NA | 4 | 1 | 1 | NA | NA | NA |
| RA 2 | 2 | 2 | 1 | 3 | 1 | 1 | 3 | 2 | 1 |
| RA 4 | 2 | 3 | 1 | 2 | 1 | 1 | 1 | 2 | 1 |
| RA 5 | 3 | 3 | 1 | 3 | 1 | 1 | 3 | 3 | 1 |
| RA 6 | 3 | 3 | 1 | 4 | 1 | 1 | 2 | 3 | 1 |
| RA 7 | 2 | 3 | 1 | 4 | 1 | 1 | 3 | 3 | 1 |
| PA 1 | 2 | 3 | 1 | 3 | 1 | 1 | 3 | 3 | 1 |
| PA 3 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 |
| PA 4 | 2 | 1 | 1 | 3 | 1 | 1 | 2 | 1 | 1 |
| PA 6 | 3 | 2 | 1 | 3 | 1 | 1 | 3 | 2 | 1 |
| PA 7 | 2 | 3 | 1 | 2 | 1 | 1 | 2 | 3 | 1 |
| PA 8 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 |
| OA 1 | NA | NA | NA | 1 | 1 | 1 | NA | NA | NA |
| OA 2 | 1 | 1 | 1 | 1 | 1 | 1 | NA | NA | NA |
| OA 3 | 1 | 1 | 1 | NA | NA | NA | NA | NA | NA |
| OA 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| OA 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| OA 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| OA 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| OA 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| OA 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| OA 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Comparison (*P*) | | | | | | | | | |
| RA/PA | 0.54 | 0.13 | 1.00 | 0.09 | 1.00 | 1.00 | 0.79 | 0.25 | 1.00 |
| RA/OA | 0.001 | 0.001 | 1.00 | 0.0005 | 1.00 | 1.00 | 0.18 | 0.003 | 1.00 |
| PA/OA | 0.0005 | 0.11 | 1.00 | 0.0005 | 1.00 | 1.00 | 0.001 | 0.14 | 1.00 |

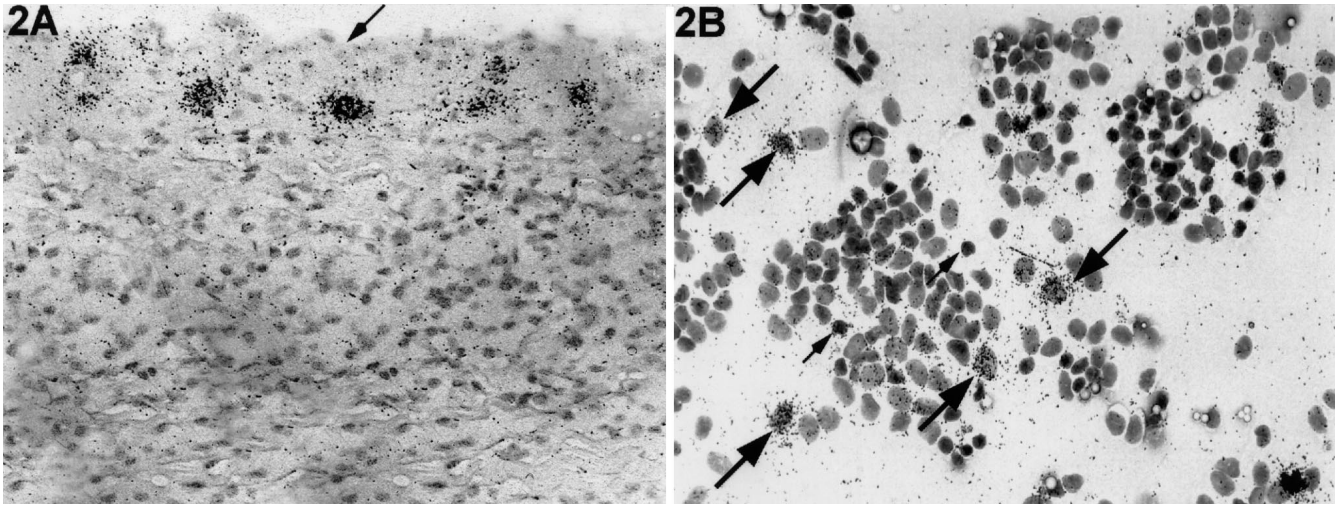


**Fig. 2A, B** GROα mRNA expression. **A** Section of synovial membrane from a psoriatic arthritis patient, showing GROα mRNA expression in the enlarged synovial lining layer (*small arrow*). Original magnification ×300 **B** Cytological preparation of psoriatic synovium. GROα mRNA expression predominates in monocytic cells (*thick arrows*). Some lymphocytic cells (*small arrows*) exhibit positive GROα mRNA signals. Original magnification ×450

tion was performed. Light microscopic examination revealed enlargement of the synovial lining layer in all RA and most PA patients. In synovial membranes of RA patients an enlarged lining layer was consistently present. A focal pattern of the infiltrate was found in 3 RA cases. The histological inflammatory score varied between 3 and 5 (Table 1). Two PA specimens showed a focal cellular infiltrate. A diffuse mononuclear cellular infiltrate was present in the psoriatic synovium in most specimens. The histological inflammatory score ranged between 1 and 4

**Table 5** Expression of MIG, GROα and RANTES mRNA in cytological preparations of synovial tissue from patients with rheumatoid arthritis (*RA*) and psoriatic arthritis (*PA*)

| Disease | Mean percentage of cells with mRNA expression (range in %) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mig | | | Groα | | | RANTES | | |
| | Monocytic | Lymphocytic | Other | Monocytic | Lymphocytic | Other | Monocytic | Lymphocytic | Other |
| RA | 75% (71–82) | 22% (15–27) | 3% (2–4) | 82% (79–85) | 14% (11–17) | 4% (3–5) | 23% (16–30) | 74% (68–82) | 3% (2–4) |
| PA | 83% (71–82) | 14% (17–21) | 3% (2–4) | 83% (82–85) | 12% (10–15) | 5% (4–6) | 22% (15–27) | 75% (71–82) | 3% (2–4) |

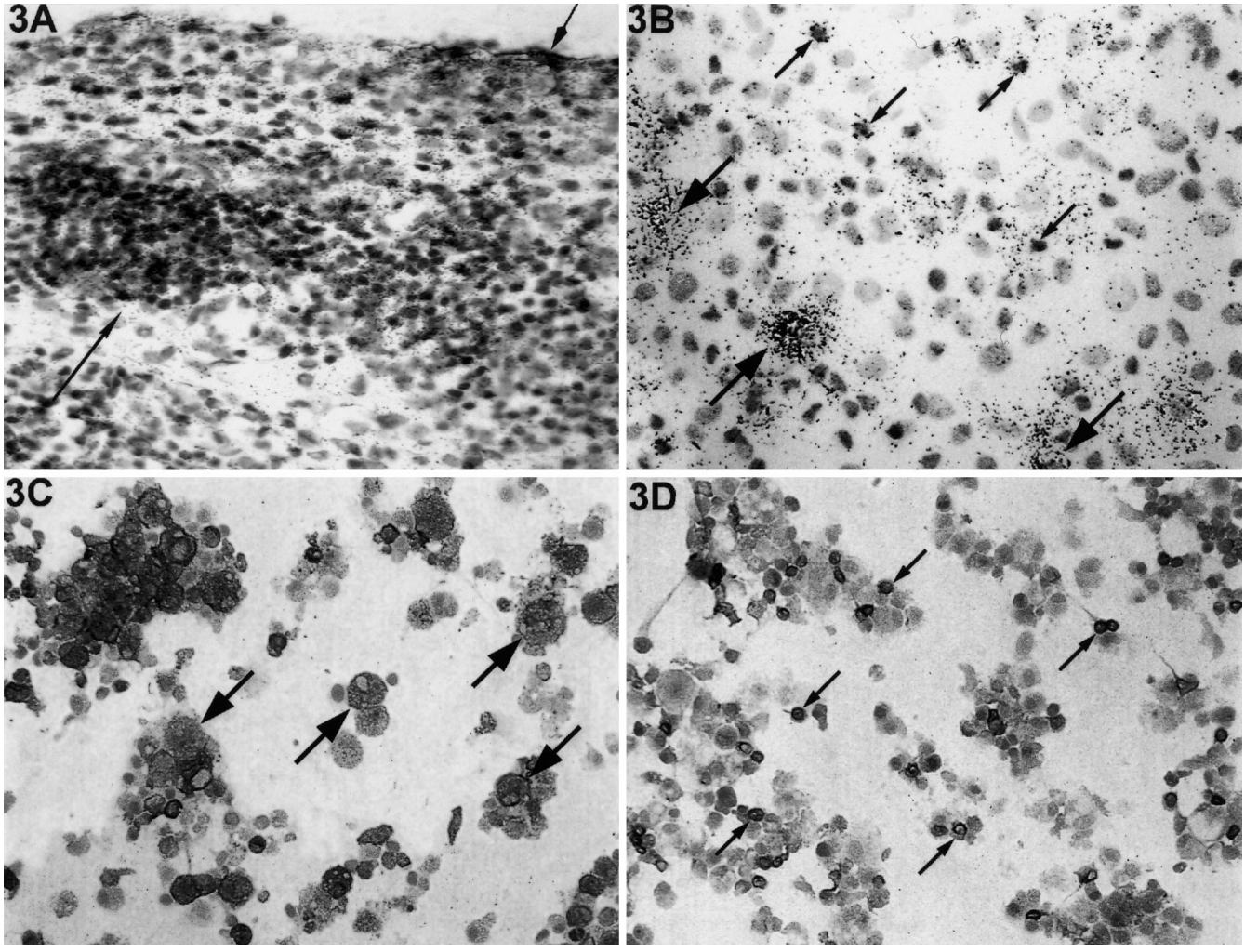


**Fig. 3A–D** RANTES mRNA expression. **A** Section of synovial membrane from a rheumatoid arthritis patient, showing RANTES mRNA expression in the enlarged synovial lining layer (*small arrow*) and cellular infiltrates (*large arrow*). Original magnification ×300. **B** Cytological preparation of psoriatic synovium. RANTES mRNA expression is seen mainly in lymphocytic cells (*small arrows*). Some monocytic cells (*thick arrows*) reveal positive RANTES signals. Original magnification ×450 **C** Cytological preparation of synovium of the same patient, showing monocytic cells (*thick arrows*) expressing macrophage antigen Ki-M 6. Original magnification ×450. **D** cytological preparation of synovium of the same patient showing lymphocytic cells (*small arrows*) expressing T lymphocyte antigen CD 3. Original magnification ×450

(Table 2). The histological scores of RA and PA did not differ significantly. OA synovium showed no enlargement of the synovial intima, and only very few mononuclear cells were present. Inflammatory scores were constantly 1 (Table 3). The histological score was significantly lower in OA compared to RA and PA ($P<0.001$).

## Clinical features

For comparison of clinical signs of inflammation with chemokine expression the disease activity in the operat-

ed joint or tendon was assessed. Local disease activity in PA patients was characterised by moderate to severe swelling and tenderness of joints or tendons in almost all cases (Table 2). The ESR was elevated in all patients (Table 2). In RA patients local disease activity was similar to PA, with mild to severe swelling and tenderness of joints or tendons (Table 1). The ESR was elevated in most RA patients (Table 1). Patients with steroid medication or small inflamed areas, e.g. tendons or finger joints, had near-normal ESR values. Patients with OA exhibited no joint swelling and mostly mild tenderness with normal ESR (Table 3). Local disease activity of RA and PA was not different, whereas OA patients had significantly less swelling than RA and PA patients ($P<0.001$).

## Discussion

Inflammatory arthropathies are characterised histologically by mononuclear cellular infiltrates and by enlargement of the synovial lining layer. Chemokines are considered as key players in the process of leucocyte diapedesis from the vasculature into tissues in inflammatory diseases [3]. There are several potentially relevant chemokines in inflammatory diseases which interact with their receptors on leucocytes and allow selective activation and chemotaxis of lymphocytes, macrophages, neutrophils and other cells in the inflammation cascade. In inflammatory arthropathies chemokines may thus contribute to the formation of inflammatory cellular infiltrates and to the enlargement of the synovial lining layer, which can lead ultimately to joint destruction. In our study, we demonstrate that the chemokines Mig, GROα and RANTES are differentially expressed in synovial tissue from PA and RA, whereas expression levels are low in OA. Notably, synovial macrophages themselves are an important source of Mig, GROα and RANTES. Owing to the different cell specificity of the highly expressed chemokines Mig, GROα and RANTES, they are involved in the trafficking of different leucocyte populations in the inflammatory reactions of PA and RA.

Our findings indicate that macrophages in the synovial intima and perivascular mononuclear aggregates and follicles are the major source of Mig in PA and RA. Our in vivo data are supported by the initial finding that in vitro Mig is produced by interferon-γ stimulated macrophages [21]. In addition, our data indicate that lymphocytes themselves produce Mig. The Mig expression pattern and the colocalisation of T cells in the inflamed synovium are suggestive that Mig mRNA is translated into functional T cell attractant protein and that Mig is an important, albeit possibly not exclusive, regulator of T cell recruitment from the peripheral blood and of migration through the synovial lining. Recent data suggest that the Th1 population predominantly expresses the Mig/IP-10 corresponding chemokine receptor CXCR3 [14]. Therefore, it is tempting to speculate that Mig expression is responsible for the recruitment of the Th1 subtype, which has been shown to be involved in RA [28]. CD4-positive cells are the dominant T cell population in rheumatoid and psoriatic synovium [17, 30]. Mig selectively attracts T lymphocytes [11, 12, 21] and recognises only one receptor, which is restricted to T lymphocytes [22]. Mig has been also detected in psoriasis [8], which may indicate a certain pathophysiological parallelism between PA and skin lesions. The high levels of Mig and the fact that Mig is almost exclusively induced by interferon-γ, as stated above, supports the concept that interferon-γ-producing Th1 cells stimulate both macrophages and lymphocytes for Mig production and further recruitment of Th1 cells.

GROα promotes T cell chemotaxis, at least in vitro [9], and thus may also stimulate migration and accumulation of T cells in vivo together with other chemokines. In rheumatoid synovium GROα was detected immunohistochemically in lining cells and macrophages [15]. It has been possible to demonstrate its production by synovial fluid mononuclear cells and its chemotactic activity for neutrophils in vitro [9, 15]. A role in regulating the ingress of leucocytes into the joint has therefore been postulated. GROα may contribute to the accumulation of neutrophils in synovial fluid due to its preferential expression in the synovial lining layer. GROα has been shown to function in vivo as a neutrophil chemotactic agent in cutaneous psoriasis [6]. In this study, GROα mRNA was detected focally in the lining layer of psoriatic and rheumatoid synovium, and not in the cellular infiltrates. In cytological preparations it was found mainly in monocytic cells. These findings may be explained by the presence of macrophages in the synovial tissue and cytological preparations. Type A synoviocytes in the lining layer, like macrophages, are part of the human mononuclear phagocyte system [5]. Chemokine expression by these type A synoviocytes may therefore play an important role in attracting leucocytes into synovial lining and fluid. The cellular influx could result in lining layer enlargement [19] and may also contribute to the increase in numbers of leucocytes in the synovial fluid of inflammatory arthropathies.

RANTES has been shown to attract monocytes and distinct T lymphocyte subsets selectively [29]. In vitro RANTES is produced by T cells [24]. In delayed-type hypersensitivity reactions RANTES was expressed by T lymphocytes and macrophages [4]. In rheumatoid synovium, RANTES gene expression was found to be present in T cells and absent in non-T cells [28]. In this study more lymphocytic than monocytic cells seemed to express RANTES in the synovium of RA and PA. RANTES has already been demonstrated by in situ hybridisation in cellular aggregates and the synovial lining layer of rheumatoid synovium [28]. By immunohistochemistry RANTES was detected in rheumatoid synovial cells [27]. Our results confirm these observations and add evidence that the same expression pattern exists in PA.

Mig and RANTES have been unequivocally demonstrated as potent T cell-attractant chemokines, whereas the situation is less clear for GROα and IL-8. According to our in situ data, RANTES and, in particular, Mig ex-

pression patterns coincide with the accumulation of T cells, whereas this spatial association could not be detected for GROα. Therefore, we conclude that for T cell infiltration in PA and RA, RANTES, as demonstrated already in a previous study [28], and, in particular, Mig are important soluble mediators. Whether IL-8, detected in inflamed synovium of patients with PA [17], contributes together with GROα for the recruitment of T cells remains to be elucidated. However, the discrepancy between the mRNA expression pattern of GROα and IL-8 and the infiltration pattern of lymphocytes rather argues against a lymphocyte-attractant property in vivo.

Osteoarthritic synovium could be shown to be largely devoid of Mig, GROα and RANTES mRNA expression. Cell-associated signals were barely detectable in this study. A lack of cellular infiltrate appears to be the cause of negligible amounts of chemokine signals. The noninflammatory nature of OA [5], which is a degenerative disorder, is supported by this observation. In contrast, PA shares most of the chemokine expression with RA. Therefore the classification of PA as inflammatory condition seems justified. MIP1β levels have been demonstrated immunohistochemically [16] to be higher in osteoarthritic synovium than in rheumatoid synovium. This protein may therefore play a role in degenerative joint pathology, but its function in a disease with hardly any cellular infiltrate must still be examined carefully.

Immunohistochemically, the production of GROα protein in OA [15] and RANTES protein in RA [27] has already been demonstrated; therefore, immunohistochemical demonstration of both chemokines at protein level was not repeated in this study. In situ hybridisation demonstrates the presence of both chemokines on mRNA level in both RA and OA, thus adding information about the microanatomical site of localisation. The demonstration of Mig, GROα and RANTES in PA is a new finding.

The histological density of cellular infiltration seems to be associated with the intensity of chemokine expression in rheumatoid and psoriatic synovium. Clinical levels of disease activity are also elevated in patients with RA and PA. In contrast, in patients with OA clinical and histological scores, and chemokine expression are low. Therefore, in inflammatory arthropathies the intensity and pattern of cellular accumulation seem to mirror the intensity and location of chemokine expression, suggesting an important role of these chemokines in the control of leucocyte migration. These laboratory findings may be reflected by clinical signs of inflammation.

It remains to be determined which cell or chemokine is the prime mover in the cascade of events leading to synovitis and ultimately to joint destruction in RA and PA. Identification of a key chemokine and its receptor may constitute the foundation of an anti-inflammatory therapy for patients suffering from RA and PA. A therapy based on interference with the chemokine system has been established in animal models of arthritis [25, 26].

In conclusion, the Mig, GROα and RANTES expression demonstrated in the cellular infiltrates and the synovial lining layer of RA and PA indicates an important role of these chemokines in regulating leucocyte traffic. Different leucocyte populations express selective chemokines and may thereby regulate the composition and localisation of the cellular infiltrate. The classification of osteoarthritis as a noninflammatory condition is supported by the very low density of the cellular infiltrate and paucity of chemokine expression. The intensity and pattern of cellular infiltration seem to depend on the degree and location of chemokine expression in human synovitis and may reflect clinical signs of inflammation.

**Acknowledgements** We would like to thank Mrs. A. Neuberger for typing the manuscript, Mrs. J. Pfeufer for technical assistance, Mrs. R. Drescher for language editing, Mr A. Schäfer for statistical advice and Mr. E. Schmitt for expert photography.

## References

1. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, Christy W, Cooke TD, Greenwald R, Hochberg M, Howell D, Kaplan D, Koopman W, Longley S, Mankin H, McShane DJ, Medsger T, Meenan R, Mikkelsen W, Moskowitz R, Murphy W, Rothschild B, Segal M, Sokoloff L, Wolfe F (1986) Development of criteria for the classification and reporting of osteoarthritis. Arthritis Rheum 29:1039–1049
2. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31:315–324
3. Baggiolini M (1998) Chemokines and leukocyte traffic. Nature 392:565–568
4. Devergne O, Marfaing-Koka A, Schall TT, Leger-Ravet MB, Sadick M, Peuchmaur M, Crevon MC, Kim T, Galanaud P, Emilie D (1994) Production of the RANTES chemokine in delayed-type hypersensitivity reactions: involvement of macrophages and endothelial cells. J Exp Med 179:1689–1694
5. Gardner DL (1992) Pathological basis of the connective tissue diseases. Lea and Febiger, Philadelphia
6. Gillitzer R, Ritter U, Spandau U, Goebeler M, Bröcker EB (1996) Differential expression of GRO-α and IL-8 mRNA in psoriasis: a model for neutrophil migration and accumulation in vivo. J Invest Dermatol 107:778–782
7. Gladmann DD (1993) Toward unraveling the mystery of psoriatic arthritis. Arthritis Rheum 36:881–884
8. Goebeler M, Toksoy A, Spandau U, Engelhardt E, Bröcker EB, Gillitzer R (1998) The C-X-C chemokine Mig is highly expressed in the papillae of psoriatic lesions. J Pathol 184:89–95
9. Jinquan T, Frydenberg J, Mukaida N, Bonde J, Larson CG, Matsushima K, Thestrup-Pedersen K (1995) Recombinant human growth-regulated oncogene-α induces T lymphocyte chemotaxis. J Immunol 155:5359–5368
10. Kellgren JH, Lawrence JS (1957) Radiological assessment of osteoarthritis. Ann Rheum Dis 16:494–501
11. Kelner GS, Kennedy J, Bacon KB, Kleyensteuber S, Largaespada DA, Jenkins NA, Copeland NG, Bazan JF, Moore KW, Schall TJ, Zlotnik A (1994) Lymphotactin: a cytokine that represents a new class of chemokine. Science 266:1395–1399
12. Kennedy J, Kelner GS, Kleyensteuber S, Schall TJ, Weiss MC, Yssel H, Schneider PV, Cocks BG, Bacon KB, Zlotnik A (1995) Molecular cloning and functional characterization of human lymphotactin. J Immunol 155:203–209
13. Kerschbaumer F, Kandziora F, Herresthal J, Hertel A, Hör G (1998) Synovektomie und Synoviorthese als Kombinationstherapie bei rheumatoider Arthritis. Orthopäde 27:188–196

14. Kim CH, Broxmeyer HE (1999) Chemokines: signal lamps for trafficking of T and B cells for development and effector function. J Leukocyte Biol 65: 6–15
15. Koch AE, Kunkel SL, Shah MR, Hosoka S, Halloran MM, Haines GK, Burdick MD, Pope RM, Strieter RM (1995) Growth-related gene product α. A chemotactic cytokine für neutrophils in rheumatoid arthritis. J Immunol 155:3660–3666
16. Koch AE, Kunkel SL, Shah MR, Fu R, Mazarakis DD, Haines GK, Burdick MD, Pope RM, Strieter RM (1995) Macrophage inflammatory protein-1β: a C-C chemokine in osteoarthritis. Clin Immunol Immunopathol 77:307–314
17. König A, Krenn V, Gillitzer R, Glöckner J, Janßen E, Gohlke F, Eulert J, Müller-Hermelink HK (1997) Inflammatory infiltrate and interleukin-8 expression in the synovium of psoriatic arthritis – an immunohistochemical and mRNA analysis. Rheumatol Int 17:159–168
18. Krenn V, Schalhorn N, Greiner A, Molitoris R, König A, Gohlke F, Müller-Hermelink HK (1996) Immunohistochemical analysis of proliferating and antigen-presenting cells in rheumatoid synovial tissue. Rheumatol Int 15:239–247
19. Krenn V, Schedel J, Döring A, Huppertz HI, Gohlke F, Tony HP, Vollmers HP, Müller-Hermelink HK (1997) Endothelial cells are the major source of sICAM-1 in rheumatoid synovial tissue. Rheumatol Int 17:17–27
20. Larsen A, Dale K, Eek M (1977) Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. Acta Radiol Diagn 18:481–491
21. Liao F, Rabin RL, Yannelli JR, Koniaris LG, Vanguri P, Farber JM (1995) Human mig chemokine: biochemical and functional characterization. J Exp Med 182:1301–1314
22. Loetscher M, Gerber B, Loetscher P, Jones SA, Piali L, ClarkLewis I, Baggiolini M, Moser B (1996) Chemokine receptor specific for IP10 and Mig: Structure, function, and expression in activated T lymphocytes. J Exp Med 184:963–969
23. Moll JMH, Wright V (1973) Psoriatic Arthritis. Semin Arthritis Rheum 3:55–78
24. Nelson PJ, Ortiz BD, Pattison JM, Krensky AM (1996) Identification of a novel regulatory region critical for expression of the RANTES chemokine in activated T lymphocytes. J Immunol 157:1139–1148
25. Nishimura A, Akahoshi T, Takahashi M, Takagishi M, Takagishi K, Itoman M, Kondo H, Takahashi Y, Yokoi K, Mukaida N, Matsushima K (1997) Attenuation of monosodium urate crystal-induced arthritis in rabbits by a neutralizing antibody against interleukin-8. J Leukocyte Biol 62:444–449
26. Plater-Zyberk C, Hoogewerf AJ, Proudfoot AEI, Power CA, Wells TNC (1997) Effect of a CC chemokine receptor antagonist on collagen induced arthritis in DBA/1 mice. Immunol Lett 57:117–120
27. Rathanaswami P, Hachicha M, Sadick M, Schall TJ, McColl SR (1993) Expression of the cytokine RANTES in human rheumatoid synovial fibroblasts. Differential regulation of RANTES and interleukin-8 genes by inflammatory cytokines. J Biol Chem 268:5834–5839
28. Robinson E, Keystone EC, Schall TJ, Gillett N, Fish EN (1995) Chemokine expression in rheumatoid arthritis (RA): evidence of RANTES and macrophage inflammatory protein (MIP)-1β production by synovial T cells. Clin Exp Immunol 101:398–407
29. Schall TJ, Bacon K, Toy KJ, Goeddel DV (1990) Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. Nature 347:669–671
30. Veale D, Yanni G, Rogers S, Barnes L, Bresnihan B, Fitzgerald O (1993) Reduced synovial membrane macrophage numbers, ELAM-1 expression, and lining layer hyperplasia in psoriatic arthritis as compared with rheumatoid arthritis. Arthritis Rheum 36:893–900