EXTENDED REPORT

Chemokine and chemokine receptor expression in paired peripheral blood mononuclear cells and synovial tissue of patients with rheumatoid arthritis, osteoarthritis, and reactive arthritis

J J Haringman, T J M Smeets, P Reinders-Blankert, P P Tak

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See end of article for authors' affiliations

Correspondence to: Professor P P Tak, Division of Clinical Immunology and Rheumatology F4-218, Department of Internal Medicine, Academic Medical Centre/University of Amsterdam, Meibergdreef 9, NL-1105 AZ Amsterdam, The Netherlands; p.p.tak@amc.uva.nl

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Background: Chemokine receptors and chemokines have a crucial role in leucocyte recruitment into inflamed tissue.
Objective: To examine the expression of an extensive number of chemokines and receptors in a unique bank of paired samples of synovial tissue (ST) and peripheral blood (PB) from patients with different forms of arthritis to assist in identifying suitable targets for therapeutic intervention.
Methods: Synovial biopsy specimens were obtained from 23 patients with rheumatoid arthritis (RA), 16 with osteoarthritis, and 8 with reactive arthritis. ST chemokine (CCL2/MCP-1, CCL5/RANTES, CCL7/MCP-3, CCL8/MCP-2, CCL14/HCC-1, CCL15/HCC-2, CCL16/HCC-4), chemokine receptor (CCR1, CCR2b, CCR5, CXCR4), and CD13 expression was analysed by immunohistochemistry and two colour immunofluorescence. Chemokine receptor expression (CCR1, CCR3, CCR5, CCR6, CCR7) on PB cells was studied by flow cytometry. Non-parametric tests were used for statistical analysis.
Results: Abundant expression of CCR1, CXCR4, and CCR5 was found in all forms of arthritis, with a specific increase of CCL5 and CCL15 in RA. CCL7, CCL8, CCL14, CCL15, and CCL16 were detected for the first time in ST. The results for PB analysis were comparable among different arthritides. Interestingly, compared with healthy controls, significantly lower expression of CCR1 ($p<0.005$) and CCR5 ($p<0.05$) by PB monocytes in the patient groups was seen.
Discussion: A variety of chemokines and receptors might have an important role in several inflammatory joint disorders. Although other receptors are involved as well, migration of CCR1+ and CCR5+ cells towards the synovial compartment may play a part in the effector phase of various forms of arthritis.

Chemokines are small chemotactic proteins that have a central role in the recruitment of leucocytes into inflamed tissue.[1–3] To date about 50 chemokines have been identified, signalling through some 20 distinct receptors.[4] Besides the ability to recruit leucocytes directly by providing a chemotactic gradient, chemokines can also activate integrins, stimulate mediator release, and modulate vascularisation, thereby playing a central part in the inflammatory process.[5]

Chemokines and chemokine receptors have been shown to be involved in a broad number of inflammatory and infectious diseases.[6] Since the discovery of their existence, targeting chemotactic proteins has been suggested as potential treatment in many disorders. Owing to the development of low molecular weight antagonists directed against chemokine receptors, which could be used as an oral treatment, the chemokine family may be an attractive therapeutic target.[7] The first clinical study using a specific CCR1 antagonist in patients with rheumatoid arthritis (RA) confirmed the potential of this approach.[8]

The analysis of synovial tissue (ST) from affected joints might assist in identifying the most important ligands and receptors in RA and other joint diseases. In particular, an analysis of their expression in both ST and peripheral blood (PB) of the same patients will provide additional evidence on their possible suitability as future therapeutic targets. Therefore, the objective of this study was to determine the expression of an extensive number of chemokines and chemokine receptors in a unique bank of paired samples of ST and PB in patients with RA, inflammatory osteoarthritis (OA), and reactive arthritis (ReA). In addition we studied the expression of CD13/aminopeptidase N, which has been described as involved in the mechanism of lymphocyte recruitment in inflamed joints of patients with RA.[9] In light of recent interest in the development of CCR1 and CCR5 blockade for treatment of RA, we had a special interest in the detection of the ligands for these receptors in the synovium. In addition, we used immunofluorescence double staining techniques to elaborate further the expression of several new chemokines on different cell types in ST.

PATIENTS AND METHODS

Patients

Forty seven patients with different forms of arthritis were included in the study. The patients fulfilled established criteria for RA ($n = 23$),[10] inflammatory OA ($n = 16$),[11] and ReA ($n = 8$).[12] The patients were followed up for at least 1 year to allow confirmation of the diagnosis. All patients had active knee arthritis defined by pain and swelling at the time of evaluation.

Most patients were treated with non-steroidal anti-inflammatory drugs. None were treated with corticosteroids

Abbreviations: APC, allophycocyanin; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; FLS, fibroblast-like synoviocytes; HRP, horseradish peroxidase; mAb, monoclonal antibody; OA, osteoarthritis; PB, peripheral blood; PBMCs, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PE, phycoerythrin; RA, rheumatoid arthritis; ReA, reactive arthritis; RT, room temperature; ST, synovial tissue

or immunosuppressive drugs at the time of the synovial biopsy. Laboratory assessments included erythrocyte sedimentation rate and C reactive protein measurements, as well as serum levels of rheumatoid factor. Clinical assessments on the day of the biopsy procedure included the Ritchie articular index[13] and number of swollen joints. All patients gave written informed consent and the study protocol was approved by the medical ethics committee.

Synovial tissue samples

An average of 20 biopsy specimens was taken from the suprapatellar pouch with a Parker Pearson needle.[14] All samples were snap frozen together in Tissue Tek OCT (Miles Diagnostics, Elkhart, IN, USA) by immersion in methylbutane (−70℃). The frozen blocks were stored in liquid nitrogen until sectioned for staining. Serial sections (5 μm) of each tissue sample, consisting of at least six different biopsy samples, were cut with a cryostat and mounted on glass slides (Start Frost, Knittelglaser, Braunschweig, Germany). The glass slides were sealed and stored at −80℃ until immunohistochemical analysis could be performed.

Antibodies

For immunohistochemical analysis the following monoclonal antibodies (mAbs) were used: anti-CD68 (EBM11, Dako, Glostrup, Denmark), anti-CD3 (SK7, Becton-Dickinson, San Jose, CA), anti-CD13 (NCL-CD13, Novocastra), anti-CCR1 (MAB145, R&D Systems Europe Ltd, Abingdon, UK), anti-CCR2b (sc-6228, Santa Cruz Biotechnology), anti-CXCR4 (MAB172, R&D), anti-CCR5 (MAB145, R&D systems), anti-CCL2/MCP-1 (sc-1304, Santa Cruz Biotechnology), anti-CCL5/RANTES (MAB278, R&D), anti-CCL7/MCP-3 (sc-1308, Santa Cruz Biotechnology), anti-CCL8/MCP-2 (sc-1307, Santa Cruz Biotechnology), anti-CCL14/HCC-1 (BAF324, R&D), anti-CCL15/HCC-2 (sc-8582, Santa Cruz Biotechnology), and anti-CCL16/HCC-4 (AF802, R&D). Goat-antimouse horseradish peroxidase (HRP, P0447, Dako) and swine-antigoat-HRP (ACI3404, Biosource (TAGO)) were used to detect bound mAbs.

For tissue immunofluorescence staining the following mAbs were used: anti-CD3 fluorescein isothiocyanate (FITC) conjugated (345763, BD), anti-CD55-FITC (M2192, CLB, The Netherlands), anti-CD68-IgG3 (M0876, Dako), anti-CCL7/MCP-3 (MAB282, R&D), anti-CCL8/MCP-2 (MAB281, R&D), and CCL15/HCC2 (MAB363, R&D). Streptavidin-TRITC (43-4314, Zymed laboratories), rabbit-anti-FITC (058, Dako), goat-antirabbit Alexa 488 (99D1-1, Molecular probes), and goat-antimouse Alexa 488 (73D1-1, Molecular probes) were used to detect bound mAbs.

For flow cytometry anti-chemokine receptor mAbs were generated in mice against their respective human proteins by Millennium Pharmaceuticals (Cambridge, MA) and generously provided. Anti-CCR1 (clone designation 2D4) and anti-CCR6 (clone designation 9H7) were mouse IgG1, anti-CCR3 (clone designation 7B11) and anti-CCR5 (clone designation 2D7) were mouse IgG2a, and anti-CCR7 (clone designation 7H12) was IgG2b. All other antibodies and controls were obtained commercially. These included anti-CD14 allophycocyanin (APC) (catalogue No 555399, Becton Dickinson (BD) Pharmingen, San Diego, CA), IgG1- R-phycoerythrin (PE) isotype control (349043, BD), IgG1-FITC isotype control (554679, BD), IgG2a-PE isotype control (349053, BD), IgG2a-APC isotype control (555576, BD), IgG2b-PE isotype control (555058, BD), and goat antimouse IgG R-PE (115-116-146, Jackson ImmunoResearch, West Grove, PA).

Immunohistochemical analysis

Serial sections were stained and sections with non-assessable tissue, defined by the absence of an intimal lining layer, were omitted before analysis. For control sections, the primary antibodies were omitted or irrelevant isotype matched antibodies were applied. Staining was performed according to a three step immunoperoxidase method, as previously described in detail.[15] After immunohistochemical staining, all coded sections were randomly analysed by computer assisted image analysis. For all markers, 18 high power fields were analysed as described earlier.[16] All sections were coded and analysed in a random order by an independent observer who was unaware of the clinical data.

Immunofluorescence tissue staining

For further quantification of chemokine expression on CD3+ T cells, CD55+ fibroblast-like synoviocytes (FLS) cells, and CD68+ macrophages, we randomly selected five ST samples from the RA group. Tissue sections were cut, stored, and fixed as described above. Sections were washed with phosphate buffered saline (PBS), and the primary antibodies or isotype matched controls were added and incubated overnight at 4℃. After incubation, sections were washed and goat-antimouse HRP antibodies were applied for 30 minutes in the dark at room temperature (RT). After washing and incubation with biotin-tyramine for 15 minutes, streptavidin-TRITC was added to the tissue sections for 30 minutes in the dark at RT. After blocking with normal mouse serum 10% in PBS, the anti-CD3, anti-CD55, and anti-CD68 antibodies were applied for 60 minutes in the dark at RT. After washing, rabbit-anti-FITC antibodies were added to the sections, which had been previously incubated with the anti-CD3-FITC and anti-CD55-FITC antibodies. Finally, after washing steps with PBS, the sections were incubated with goat-antirabbit Alexa 488 for the sections previously incubated with anti-CD3 and anti-CD55, and with goat-antimouse Alexa 488 for the sections previously incubated with anti-CD68. After a 30 minute incubation at RT the slides were washed and cover slipped with Vectashield (Vector). The sections were examined under a fluorescent photomicroscope. Two observers independently quantified the number of double staining positive cells by manual counting. On average, between 100 and 200 cells which were positive for CD3, CD55, or CD68 were counted by each observer. The mean percentage of double staining cells with the anti-chemokine antibodies was calculated from the results of the two observers.

Flow cytometry

PB mononuclear cells (PBMCs) were obtained at the same day as the ST samples from the same patient groups, as well as from five healthy controls, and were isolated from PB samples by Ficoll-Hypaque gradients and directly stored in liquid nitrogen. Previously it was shown that isolated PBMCs can be frozen and stored in liquid nitrogen without affecting chemokine receptor expression on CD14+ monocytes. Chemokine receptor expression on these cells, measured by flow cytometry, was comparable with the chemokine receptor expression in paired fresh whole blood samples (Haringman JJ *et al*, unpublished data).

The vials were removed from the liquid nitrogen storage and thawed at room temperature until only a small clot was still present. The contents of each vial were transferred immediately into 10 ml tubes and then slowly filled with Dulbecco's modified Eagle's medium (DMEM) +20% fetal calf serum (FCS). Tubes were centrifuged for 10 minutes at 500 *g* (1700 rpm) at 4℃. The supernatant was discarded and the cells resuspended. Next 10 ml DMEM + 10% FCS was added to each tube and mixed to make a homogeneous PBMC suspension. Cells were counted and resuspended at a concentration of 2×10^6/ml with DMEM + 10% FCS.

After thawing, the cells were incubated for 30 minutes at 4℃ in the dark with primary antibodies directed against

Table 1 Clinical and demographic data of 23 patients with RA, 16 patients with OA, and 8 patients with ReA

Characteristic	RA (n = 23)	OA (n = 16)	ReA (n = 8)
Sex (M/F)	9/14	2/14	6/2
Age (years)	58 (3) {24–76}	72 (2) {54–81}	33 (4) {16–47}
Disease duration (months)	7 (1) {2–12}	–	5 (2) {1–18}
Total Ritchie score	10 (1) {3–26}	4 (1) {0–15}	1 (1) {0–5}
Swollen joint count	8 (1) {3–18}	2 (1) {0–9}	2 (0) {1–4}
ESR (mm/1st h)	63 (7) {13–125}	33 (6) {10–89}	15 (4) {3–28}
CRP (mg/l)	51 (9) {11–214}	21 (7) {3–112}	14 (7) {3–50}
RF (+/–)	13/10	0/16	0/8

The data are shown as the mean (standard error of the mean) {range}.
ESR, erythrocyte sedimentation rate; CRP, C reactive protein; RF, rheumatoid factor.

CCR1, CCR3, CCR5, CCR6, and CCR7, or appropriate IgG isotype controls. After several washing steps, the cells were incubated with goat-antimouse IgG R-PE for 30 minutes. After washing steps APC conjugated CD14 mAbs were added for 30 minutes at 4°C in the dark. Cells were analysed using a FACSCaliber flow cytometer and CellQuest software (BD Biosciences) and the percentages of positive monocytes were determined. Monocytes were differentiated by characteristic side and forward light scatter properties and confirmed by CD14 staining. The threshold level was based on the maximum staining of a matched isotypic antibody with irrelevant specificity used in the same concentration. Isotype control antibody bound to <1% of cells, and results are reported as the proportion of cells with fluorescence signals above the cut off point, as defined by the isotype controls.

Statistical analysis

Patient data were analysed using non-parametric tests (Kruskal-Wallis H test and the Mann-Whitney U test) for the comparison of chemokine/receptor expression between the three diagnostic groups. Values of $p<0.05$ were considered significant.

RESULTS

Clinical and demographic features

Table 1 presents the clinical and demographic characteristics (obtained at the time of sample collection). On average, patients in the group with inflammatory OA were older than the patients in the other two groups. Thirteen of the 23 patients with RA were rheumatoid factor positive, whereas none of the patients from the OA and ReA group were rheumatoid factor positive.

Table 2 Immunohistological features of synovial tissue from patients with RA, OA, and ReA for CD3+ T lymphocytes, CD68+ macrophages, CD13+ cells, CCR1+ cells, CCR2b+ cells, CCR5+ cells, CXCR4+ cells

	RA (n = 23)	OA (n = 16)	ReA (n = 8)
CD3+ T lymphocytes*	331 (83)	46 (13)	75 (26)
CD68+ macrophages	1015 (178)	383 (111)	601 (256)
CD13+ cells	640 (172)	381 (122)	547 (236)
CCR1+ cells	1232 (231)	1274 (182)	955 (317)
CCR2b+ cells	262 (71)	114 (37)	266 (152)
CCR5+ cells	1335 (315)	982 (248)	730 (201)
CXCR4+ cells	815 (202)	784 (282)	706 (353)

The data represent the mean number of positive cells per 2.1 mm^2 (standard error of the mean).
*Kruskal-Wallis test $p<0.05$, Mann-Whitney test, comparison between RA and OA: $p<0.05$, comparison between RA and ReA: not significant, comparison between OA and ReA: not significant.

Immunohistochemical analysis

Chemokine receptors

Slides were negative when the primary antibody was omitted or irrelevant antibodies were applied. All chemokine receptors could be detected in the ST in all groups. Table 2 and fig 1A show the mean scores (standard error of the mean (SEM)) and representative examples of chemokine receptor expression in ST, respectively. On average, there was abundant ST expression of CCR1, CCR5, and CXCR4 in patients with RA, OA, and ReA. CD13 and CCR2b were also present in ST of all patients, although at a lower expression level.

Immunohistological analysis showed no statistically significant differences in chemokine receptor expression between RA, inflammatory OA, and ReA, indicating that these chemokine receptors are up regulated in all forms of arthritis.

Chemokines

All chemokines could be detected in inflamed synovium. Table 3 gives the mean scores (SEM) for the chemokine markers investigated in this study and fig 1B shows representative examples of chemokine expression in ST. CCL2/MCP-1, CCL5/RANTES, CCL8/MCP-2, and CCL15/HCC-2, especially, were abundantly expressed in the ST of patients with RA. The differences between RA and the other two groups did not reach statistical significance for CCL2/MCP-1, CCL7/MCP-3, CCL8/MCP-2, CCL14/HCC-1, and CCL16/HCC-4. The expression of CCL5/RANTES was higher in RA than in the OA and ReA groups. Similarly, CCL15/HCC-2 expression was significantly increased in RA (table 3).

Immunofluorescence double staining

As some of the investigated chemokines are described here for the first time in ST of patients with RA, we performed double immunofluorescence to phenotype positive cells. The expression of CCL7/MCP-3, CCL8/MCP-2, and CCL15/HCC-2 by T cells, FLS, and macrophages, respectively, was determined. None of the CD3+ T cells showed coexpression with CCL7/MCP-3 or CCL8/MCP-2, while on average 57 (4)% of the CD3+ lymphocytes showed coexpression with CCL15/HCC-2 (fig 2). The immunohistochemical analysis indicated that CCL7/MCP-3 and CCL8/MCP-2 were mainly expressed in the intimal lining layer. Double immunofluorescence showed that 45 (15)% and 50 (12)% of the CD55+ FLS coexpressed CCL7/MCP-3 and CCL8/MCP-2, respectively. Of the CD55+ FLS, 72 (11)% were positive for CCL15/HCC-2. Of the CD68+ macrophages, on average, 27 (12)% coexpressed CCL7/MCP-3, 25 (4)% CCL8/MCP-2, and 55 (12)% of these cells coexpressed CCL15/HCC-2 (fig 2).

Flow cytometry analysis

For all patients as well as for five healthy controls we evaluated the expression of CCR1, CCR3, CCR5, CCR6, and

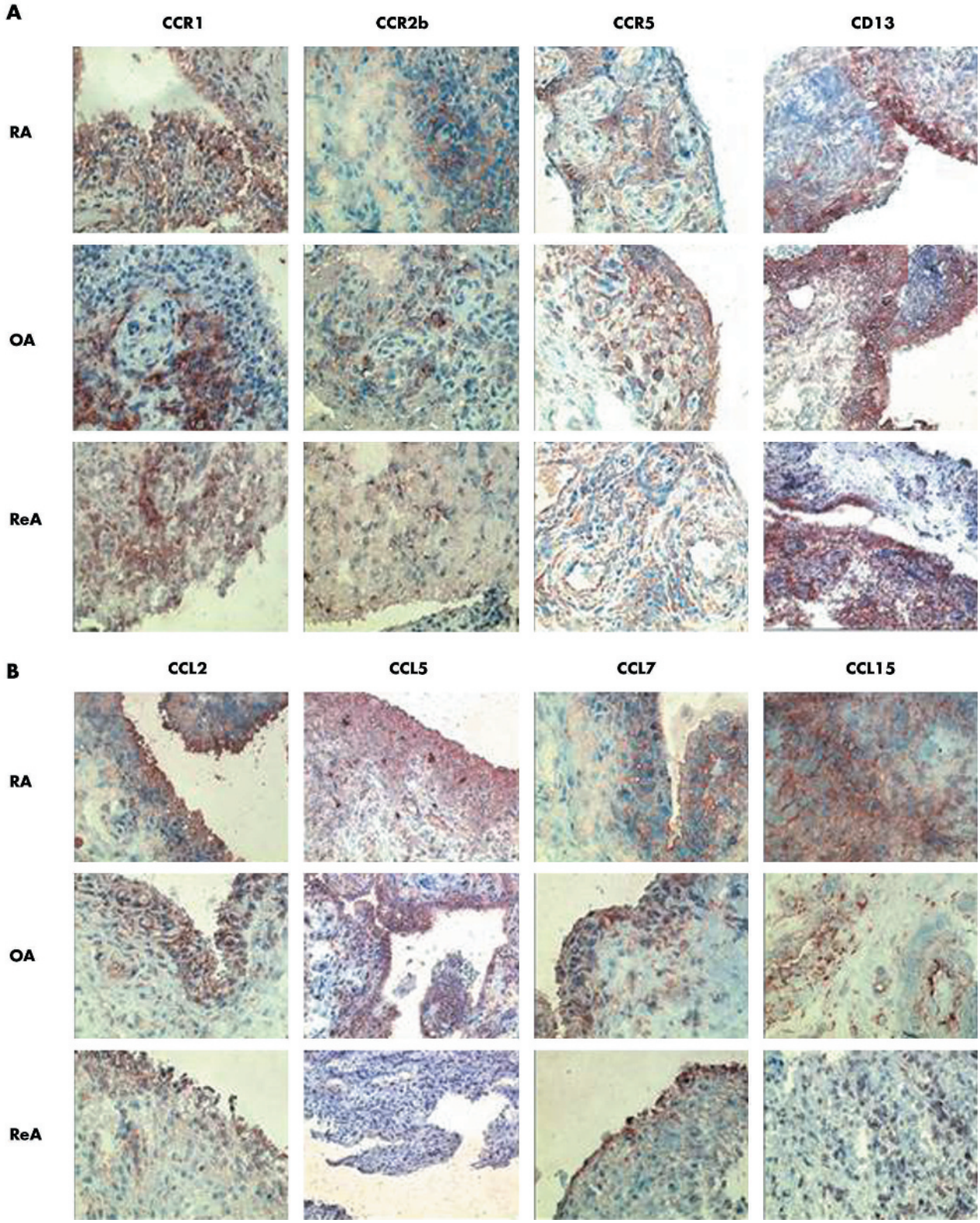

Figure 1 (A) Expression of the chemokine receptors CCR1, CCR2b, CCR5, and the CD13/aminopeptidase N in the ST of patients with RA, OA, and ReA. Original magnification ×400, ×400, ×400, ×200 respectively. (B) Representative expression of the chemokines CCL2/MCP-1, CCL5/RANTES, CCL7/MCP-3, and CCL15/HCC-2 in the ST of patients with RA, OA, and ReA. Original magnification ×400, ×200, ×400, ×400, respectively.

CCR7 on CD14+ PB monocytes with antibodies available for the detection of chemokine receptors in PB (fig 3). Chemokine receptor expression could be detected in all samples. The results of chemokine receptor expression by CD14+ monocytes were comparable among the different disease groups. Of interest, when the results were compared with the healthy controls we observed significantly lower expression of CCR1 in the arthritis groups ($p<0.005$).

Table 3 Immunohistological features of synovial tissue from patients with RA, OA, and ReA for CCL2/MCP-1, CCL5/RANTES, CCL7/MCP-3, CCL8/MCP-2, CCL14/HCC-1, CCL15/HCC-2, and CCL16/HCC-4

	RA (n = 23)	OA (n = 16)	ReA (n = 8)
CCL2	6867 (2823)	1020 (343)	687 (379)
CCL5 *	11202 (2360)	8759 (2930)	2081 (1181)
CCL7	1471 (650)	1555 (758)	1208 (663)
CCL8	10653 (3877)	3819 (1384)	1664 (716)
CCL14	1072 (313)	435 (314)	1071 (999)
CCL15†	4993 (1601)	1738 (573)	1253 (919)
CCL16	2213 (1137)	1674 (801)	1323 (650)

The data represent the mean integrated optical density per 2.1 square mm^2 (SEM).
*Kruskal-Wallis test $p<0.05$, Mann-Whitney test, comparison between RA and OA: not significant, comparison between RA and ReA: $p<0.05$, comparison between OA and ReA: $p<0.05$; †Kruskal-Wallis test $p<0.05$, Mann-Whitney test, comparison between RA and OA: not significant, comparison between RA and ReA: $p<0.05$, comparison between OA and ReA: not significant.

Patients in the RA group, on average, also showed lower expression of CCR5 than healthy controls ($p<0.05$) (fig 3).

DISCUSSION

This study investigated the expression of a broad variety of chemokines and chemokine receptors in paired samples of ST and PB from patients with RA, inflammatory OA, and ReA. Of interest, the percentages of CCR1 and CCR5 positive monocytes were decreased in PB of patients with RA compared with normal subjects. There was abundant expression of CCR1 and CCR5 in the ST of these patients, indicating up regulation of these receptors and/or accumulation of CCR1 and CCR5 positive cells in the inflamed synovium.

Chemokines and chemokine receptors are important mediators of leucocyte trafficking in inflammatory disorders and many family members may be potential targets for biological intervention in a variety of diseases.[17] As many of the chemokines and receptors have a role in cell migration and inflammation, it is difficult to predict which ligands or receptors are the best candidates to target. Studying their expression in paired ST and PB of the same patients will assist in the process of selecting the best candidates for therapeutic intervention.

Both CCR1 and CCR5 have been shown to have a specialised role in the recruitment of monocytes and Th1-type T cells under inflammatory conditions.[18] In RA, CCR1 and CCR5 have been implicated as potential therapeutic targets as they seem strongly involved in monocyte and T lymphocyte recruitment towards the joints.[19] Although both cell types are intimately involved in the pathogenesis of RA, it was recently shown that ST macrophages, in particular, are related to clinical signs and symptoms.[20–23] Both CCR1 and CCR5 have been shown to be expressed on a large number of ST macrophages. Interference with the migration of these cells by cytokine receptor blockade is therefore suggested as a new therapeutic approach to reduce synovial inflammation. The only published study on chemokine blockade in patients so far indicated that short term treatment with a specific CCR1 antagonist resulted in an evident reduction in the number of ST macrophages.[8]

The results of our study confirm the expression of both CCR1 and CCR5 in the ST and PB of patients with RA and other arthritides, but also show that other chemokine receptors and chemokines are involved as well. Although these data do not prove that these chemokine receptors have a functional role in arthritis, it can be expected that they are involved in the inflammatory process based on reported functional studies.[18]

Except for CCL5/RANTES and CCL15/HCC-2, which were present at higher levels in RA ST, there were on average no significant differences in the expression of the analysed chemokine receptors and chemokines in RA compared with the disease controls, indicating that chemokines and chemokine receptors are not uniquely restricted to inflammation in RA. Thus based on these expression data, chemokine blockade might not only be a potential treatment for patients with RA but also for other inflammatory joint disorders, because this approach is directed at common final pathways.

In addition to chemokine receptors, we investigated the expression of many of their ligands in the synovium. Our results confirm earlier reports that there is abundant expression of CCL2/MCP-1, mainly in the intimal lining layer, and CCL5/RANTES, diffusely expressed in ST, especially in patients with RA.[24 25] CCL7/MCP-3 and CCL8/MCP-2, which are ligands of both CCR1 and CCR2, were expressed abundantly in the ST both of patients with RA and disease controls. These ligands are structurally similar to CCL2/MCP-1 and influence migration of especially lymphocytes and monocytes.[26] This is the first description of CCL7/MCP-3 and CCL8/MCP-2 in ST. The expression of CCL7/MCP-3 and CCL8/

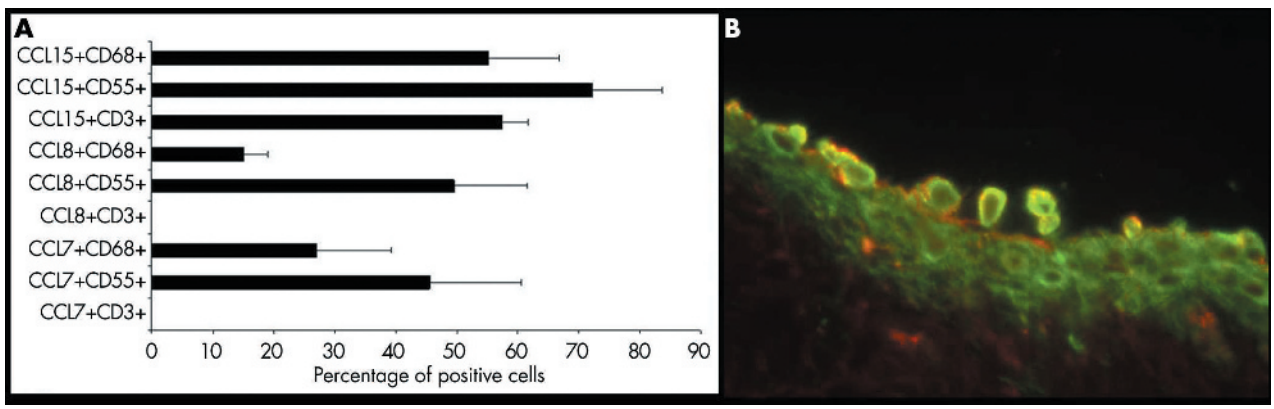

Figure 2 (A) Mean (SEM) percentage of double staining CD3+ T cells, CD55+ FLS, and CD68+ macrophages with the chemokines CCL7/MCP-3, CCL8/MCP2, and CCL15/HCC-2 in the synovium of five patients with RA. (B) Representative example of immunofluorescence double staining of CD55+ FLS (green) and CCL7/MCP-3 (red) in the ST of a patient with RA (original magnification ×1000).

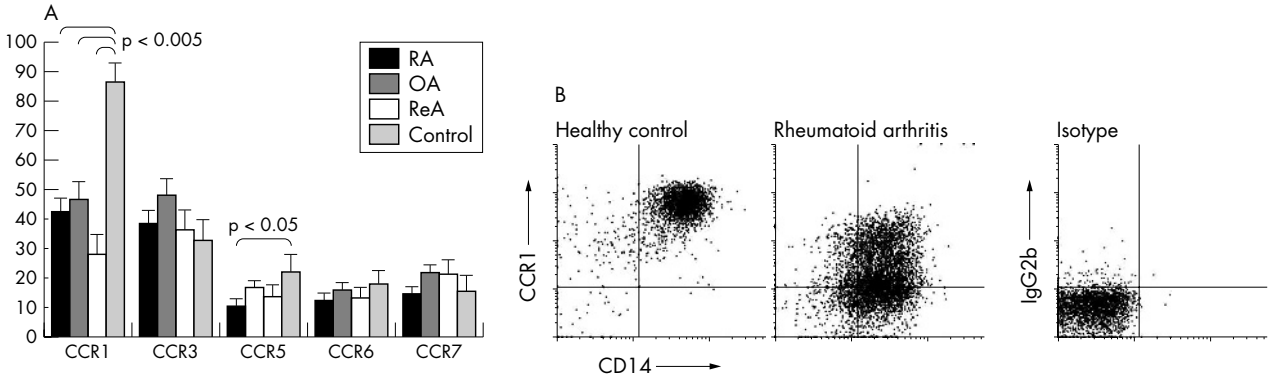

Figure 3 (A) Percentages (SEM) of CD14+ PB monocytes expressing the chemokine receptors CCR1, CCR3, CCR5, CCR6, and CCR7 in 20 patients with RA, 16 with OA, 8 with ReA, and 5 healthy controls. (B) Representative flow cytometry dot plots showing chemokine receptor 1 (CCR1) expression on CD14+ cells in healthy control and RA PB together with the isotype matched control (IgG2b). CD14+ monocytes were defined by APC and side scatter properties. A gate was drawn around the CD14+ population and CCR1 positively staining cells were chosen by the PE fluorescence over isotype matched control (IgG2b).

MCP-2 resembles that of CCL2/MCP-1, with marked expression by FLS and macrophages in the intimal lining layer.

In addition, this study shows for the first time the expression of CCL14/HCC-1, CCL15/HCC-2, and CCL16/HCC-4, ligands of CCR1, in the inflamed synovium. CCL14/HCC-1 differs from most chemokines as it is present in high concentrations in human plasma.[27] CCL14/HCC-1 is a low affinity agonist of CCR1, which is converted into a high affinity agonist of CCR1 (and CCR5) by serine proteases.[28] CCL15/HCC-2, which binds to CCR1 and CCR3, has a chemoattractant role for neutrophils, lymphocytes, and monocytes.[29] Besides having chemotactic and proinflammatory effects, CCL15/HCC-2 is also known to promote homoeostasis and was reported to be expressed only in the gut and the liver.[30 31] CCL16/HCC-4 is another chemokine expressed in the liver, which is also known to be up regulated in colonic biopsy samples from patients with ulcerative colitis.[32] It has been suggested that this chemokine is an effective inducer of cell adhesion and it has been shown to activate angiogenic programmes in vascular endothelial cells.[33 34] The results of our study show that these inflammatory mediators are present in the ST of all patient groups. CCL15/HCC-2 was expressed at higher levels in the ST of patients with RA. Immunofluorescence double staining showed that more than half of the CD3+ T cells and CD68+ macrophages and more than 70% of the CD55+ FLS coexpressed CCL15/HCC-2. This indicates that CCL15/HCC-2 may be an important contributor to cell migration in synovitis.

Taken together, the data indicate that activation of the chemokine network represents a pivotal common final pathway in synovial inflammation. The strong expression of chemokine receptors in the ST may be explained by up regulation of the receptors or increased migration of cells expressing these receptors towards the site of inflammation, or a combination of both. The abundant expression of CCR1 and CCR5 in rheumatoid ST, in combination with their decreased expression on monocytes from paired PB, suggests a possible role in the migration of mononuclear cells from the PB towards the joints. Both monocytes and lymphocytes may participate in this process.

In addition, based on our data, CCR1 also appears to have a role in other joint diseases. Interference with this mechanism may result in decreased infiltration of leucocytes into the joints and a subsequent improvement in signs and symptoms, as recently was described for the effects of a specific CCR1 antagonist in patients with RA.[8] Although other receptors and ligands are involved as well, blockade of CCR1 and CCR5 may be a potentially effective treatment for a variety of arthritides. This study provides the rationale for current and future functional studies and, subsequently, for clinical trials investigating the true potential of their application as therapeutic targets in patients.

Authors' affiliations

J J Haringman, T J M Smeets, P Reinders-Blankert, P P Tak, Division of Clinical Immunology and Rheumatology, Academic Medical Centre/ University of Amsterdam, The Netherlands

Competing interests: None.

JJH contributed to the experiments and was responsible for data analysis and interpretation and wrote the manuscript.

TJMS and PRB were responsible for both the set up and performance of the experiments.

PPT was responsible for the planning of the work and contributed to data analysis, interpretation, and writing up.

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