

Plasma Level of CXC-Chemokine CXCL12 Is Increased in Rheumatoid Arthritis and Is Independent of Disease Activity and Methotrexate Treatment

IB HANSEN, TORKELL ELLINGSEN, NETE HORNUNG, JØRGEN H. POULSEN, TINE LOTTENBURGER, and KRISTIAN STENGAARD-PEDERSEN

ABSTRACT. *Objective.* Several actions of the chemokine CXCL12 have potential relevance for rheumatoid arthritis (RA). Interaction with CXCR4, the unique receptor for CXCL12, stimulates angiogenesis, mononuclear cell trafficking into the joints, lymphoid-tissue-like rearrangement of T cells within the synovium, and chondrocyte release of cartilage-degrading metalloproteinases. We investigated the level of CXCL12 in plasma (p-CXCL12) as a marker of RA diagnosis, RA disease activity, and response to methotrexate (MTX) treatment.

Methods. A prospective study including 36 patients with RA (ACR criteria) of at least 6 months' duration, and 50 sex and age matched healthy controls. ELISA for CXCL12 was performed on plasma prior to and after 16 and 28 weeks of MTX treatment in the patients with RA and once in controls.

Results. The p-CXCL12 was 1855 ± 145 pg/ml in RA patients and 1273 ± 79 pg/ml in controls ($p < 0.001$). During the 28 weeks of MTX treatment, the ACR disease activity variables decreased, whereas the p-CXCL12 level remained constant and increased. P-CXCL12 was not correlated to any ACR disease activity variable at any time ($p > 0.05$).

Conclusion. Patients with RA had a significantly and constantly increased p-CXCL12 level compared to controls. The p-CXCL12 level was independent of any ACR disease activity variables, as well as response to MTX treatment. (J Rheumatol 2006;33:1754–9)

Key Indexing Terms:

STROMAL CELL-DERIVED FACTOR-1 α
RHEUMATOID ARTHRITIS

CXCL12

CXCR4
CHEMOKINES

Rheumatoid arthritis (RA) is a chronic, inflammatory disease, mainly affecting synovial joints. The cause of the inflammation and its primary confinement to the joints is unknown. A possible contributor is the complex system of cytokines and chemokines exhibiting regulatory actions in the inflammatory system¹⁻⁵. Chemokines are major regulators of cell trafficking from blood to tissue⁶⁻⁸. The production and secretion of

CXCL12, a CXC chemokine with many actions of potential importance for the development of RA, has been demonstrated in joint endothelial cells^{9,10}. It causes migration of selected bypassing mononuclear cells^{4,11-15}, especially CD4+ T cells, which are the dominating cells in the joints of patients with RA^{14,16}. The coupling of CXCL12 to CXCR4 further causes T cell accumulation in the synovium of RA patients through both inhibition of synovial T cell apoptosis¹⁷⁻¹⁹ and active retention of T cells in the RA synovium²⁰. This ligand-receptor pair also stimulates the lymphoid tissue-like rearrangement of T cells, primarily of the CD4+CD45RO+ memory type, in chronic inflammatory synovial tissue^{11,20-22}. Within the joint, the fibroblast-like synoviocytes (FLS) produce and secrete CXCL12²³⁻²⁵, and CXCL12-CXCR4 interaction elicits stimulation of angiogenesis^{9,10,26-31}, which is important for the uncontrolled growth of pannus in RA^{32,33}. CXCR4, but not CXCL12, is highly expressed on chondrocytes, and coupling to CXCL12 causes release of metalloproteinases (MMP), which are cartilage matrix-degrading enzymes³⁴⁻³⁷. All actions of the CXCL12-CXCR4 system described here are most probably highly specific, because, unlike most other CXC chemokines, CXCL12 binds to only one receptor, CXCR4^{13,38}, even though there has been one report that CXCL12 also binds to CXCR7³⁹. Concerning patients with RA, there are no data on an association between CXCL12 and

From the Department of Rheumatology, Aarhus University Hospital, Aarhus, Denmark; Department of Clinical Biochemistry, Randers Hospital, Randers, Norway; Department of Clinical Biochemistry, Holbaek Hospital, Holbaek; and King Christian X's Rheumatism Hospital, Graasten, Denmark.

Supported by the Danish Rheumatism Association, Poul Martin Christiansen's and Wife's Foundation, The Harboe Foundation, A.P. Moeller's and Wife Chastine McKinney Moeller's Foundation, Consul Johannes Fogh-Nielsen's and Mrs. Ella Fogh-Nielsen's Legacy, and Helga and Peter Korning's Foundation.

I. Hansen, MD, PhD; T. Ellingsen, MD, PhD, Department of Rheumatology, Aarhus University Hospital; N. Hornung, MD, PhD, Department of Clinical Biochemistry, Randers Hospital; J.H. Poulsen, MD, Chief Physician, Department of Clinical Biochemistry, Holbaek Hospital; T. Lottenburger, MD, PhD, King Christian X's Rheumatism Hospital; K. Stengaard-Pedersen, MD, DMSc, Professor, Chief Physician, Department of Rheumatology, Aarhus University Hospital.

Address reprint requests to Dr. I. Hansen, Aarhus University Hospital, Reumatologisk afdeling, Noerrebrogade 44, bygning 3, 8000 Aarhus C, Denmark. E-mail: pip@dadlnet.dk

Accepted for publication April 17, 2006.

disease activity. The concentration of CXCL12 in synovial fluid (SF) from patients with RA has been measured more than 10-fold higher compared to that in healthy controls and almost 3-fold higher than that in patients with osteoarthritis (OA)⁴⁰. Recently, CXCL12 was shown to be expressed in RA synovial tissue despite a marked clinical treatment response to anti-tumor necrosis factor- α (anti-TNF- α)⁴¹.

The aim of our study was to test if the level of CXCL12 in plasma was correlated to RA per se, to disease activity, and to response to methotrexate (MTX) treatment.

MATERIALS AND METHODS

Patients. The study was carried out at the Department of Rheumatology, Aarhus University Hospital, Aarhus, Denmark. Participation was based on patients' written consent according to the Declaration of Helsinki⁴², and the study was approved by the local ethical committee.

Eligible for inclusion were 36 patients with RA fulfilling the American College of Rheumatology (ACR) diagnostic criteria⁴³. Disease duration was > 6 months, with a mean of 13.3 years (range 0.5–45). The distribution by sex was 25 women and 11 men, with a mean age of 55.1 years (range 32–77). All 36 patients were MTX-naive, 11 patients were DMARD-naive, and 25 patients had taken disease modifying antirheumatic drugs (DMARD): one DMARD (17 patients), 2 (3 patients), or at least 3 DMARD (5 patients) including salazopyrin, myochrysin, penicillamine, auranofin, and hydroxychloroquine. These 25 patients underwent a 4-week washout period prior to start with MTX treatment. The initial dose of MTX was 7.5 mg once weekly and, with signs of disease activity at the monthly followup, the weekly dose was escalated by 2.5 mg. The mean dose of MTX for the 36 RA patients after 0, 16, and 28 weeks was 7.5, 8.5, and 9.8 mg, respectively, and a dose range of 5–15 mg. Oral prednisone was administered to 17 patients for the whole 28-week study period at a mean dose of 9.7 mg (2.5–30 mg/day). No patient received intraarticular steroid. Nonsteroidal antiinflammatory drug use was allowed.

After MTX start, disease activity was assessed in all patients by the same physician at Weeks 0, 16, and 28. The assessing physician was blinded to the physician in charge of MTX treatment and dose adjustment for disease activity, adverse events, and the study objective of disease remission. Disease activity was assessed by the swollen joint count (a modified ACR 28 index with addition of ankles and 10 metatarsophalangeal joints adding up to a total of 40 joints⁴⁴, the tender joint count (the same count with addition of the hips, thus a total of 42), physician's global assessment of disease activity on a 5-point numeric rating scale (NRS), patient's global assessment of disease activity on a 10 point NRS, patient's HAQ score (Stanford Health Assessment Questionnaire)⁴⁵ of functional activity (from 0 to 3), and finally, pain score on a 10 point NRS. Response to MTX treatment was evaluated by the ACR criteria of improvement^{46,47}.

Plasma samples from 50 healthy control subjects were collected; their female to male ratio was 1:1 and their mean age was 47 years (range 22–65).

Plasma. For several reasons, plasma, rather than synovial fluid and synovial tissue, was chosen for CXCL12 analysis. First, to potentially serve as a marker for diagnosis, disease activity, and response to MTX treatment in RA, the analyzed medium should be easily accessible, reproducible (serial measurements over 28 weeks), and part of daily clinical handling of the patients. Second, plasma samples are far more acceptable for the participants. Third, pilot studies had shown increased levels in RA patients compared to healthy controls.

Plasma from RA patients and healthy controls was isolated from heparinized blood samples after centrifugation and frozen in aliquots at -80°C for later analysis.

To check for diurnal variation in plasma-CXCL12 (p-CXCL12) a total of 29 patients with RA had 4 blood samples taken within 24 hours at time 0, 6, 12, and 24 hours.

ELISA analysis. CXCL12 ELISA was performed using kits from R&D

Systems, Abingdon, UK (catalog no. DSA00). All samples were run in doublets and, if the coefficient of variation (CV) was > 10%, the samples were rerun.

The assay was checked for intraassay (CV = 4.5%) and interassay variation (CV = 12.5%), spike recovery, linearity (dilution), and cross-reactivity with other isotopes. All validation results were in accord with those reported by R&D Systems.

Statistics. Spearman's rank-order correlation analysis was applied to the relationship between p-CXCL12 and each single RA activity variable. Student's t test was used to compare p-CXCL12 in the 50 healthy controls to that of the 36 patients with RA. This test was also applied on the comparison of p-CXCL12 levels at Weeks 0, 16, and 28. P values < 0.05 were considered significant.

RESULTS

In the 36 patients with long-standing RA, p-CXCL12 was increased compared to that in the 50 healthy controls (Figure 1; $p < 0.001$). The p-CXCL12 level remained constant over time in the RA patients (Figure 2) and showed no correlation to ACR disease activity variables before start of MTX treatment (Week 0) or after 16 and 28 weeks (Table 1, Figure 3) [correlation coefficients (r values) between 0.32 and 0.02, corresponding to p values between 0.055 and 0.912]. The plots of RA disease activity versus p-CXCL12 level after 16 and 28 weeks of MTX are not shown, but followed the same patterns as in Figure 3 and showed no significant correlation. Arbitrarily, RA patients were grouped according to p-CXCL12 levels: increase > 10% (12.3–50.9%), decrease > 10% (14.8–43.8%), constant over the 28-week study period, and compared with RA disease activity; no differences were observed ($p = 0.389\text{--}0.967$). The 36 RA patients were divided

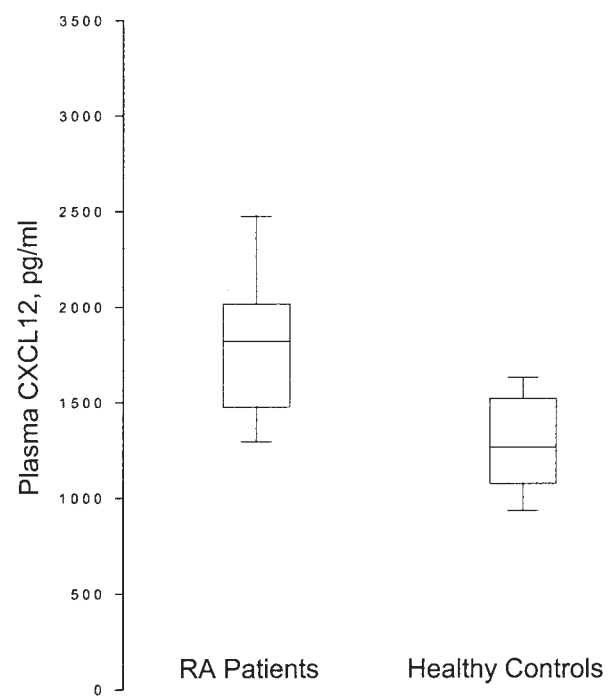


Figure 1. Plasma CXCL12 level in patients with RA ($n = 36$) and healthy controls ($n = 50$). Lines in boxes indicate mean values, their height representing 25th and 75th quartiles; 10th and 90th percentiles are also shown.

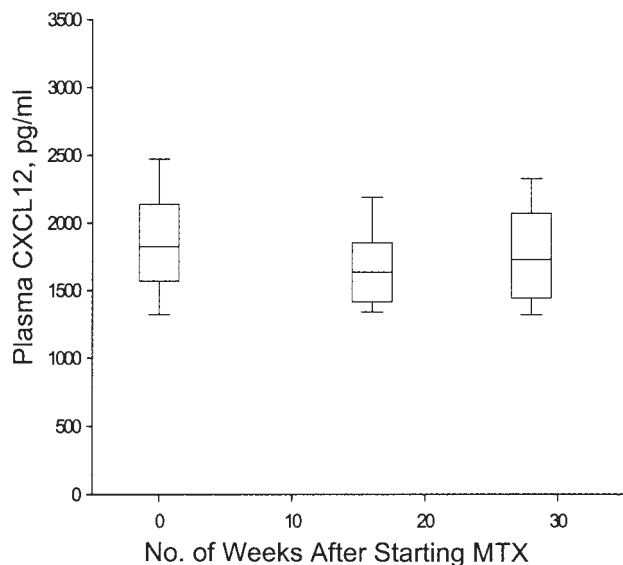


Figure 2. P-CXCL12 in 36 patients with long-standing RA before and 16 and 28 weeks after start with MTX treatment. Lines in boxes indicate mean values, their height representing 25th and 75th quartiles; 10th and 90th percentiles are shown.

into 3 groups of 12 each, based on the level of p-CXCL12 prior to start with MTX. From the one-third of patients with the highest p-CXCL12 values (> 2000 pg/ml, representing values exceeding 95% CI of the mean) the values for each of the ACR-defined RA activity variables were compared to the same values from the one-third of patients with the lowest p-CXCL12 values (< 1710 pg/ml, representing values below the 95% CI of the mean). No RA disease activity variable levels in these 2 groups differed from each other (p values between 0.213 and 0.546).

Concerning the response to MTX treatment, 11 patients (30%) had at least ACR20 response 28 weeks after MTX start, 19 patients (53%) were nonresponders, and 6 patients (17%) had no Week 28 response data for reasons unrelated to RA activity. Responders ($>$ ACR20) and nonresponders to MTX treatment after 28 weeks had equal p-CXCL12 levels ($p = 0.483$). To test whether oral prednisone influenced the p-

CXCL12 level, we compared levels in the 17 patients who were receiving prednisone to 19 patients who were not, but found equal values in the 2 groups ($p = 0.949$).

We found no diurnal fluctuations in the p-CXCL12 level when blood samples were taken after 0, 6, 12, and 24 hours from each of 29 patients with RA.

In summary, a high p-CXCL12 level was associated with the RA diagnosis per se. The p-CXCL12 remained high over time and was independent of disease activity including inflammatory variables and response to MTX treatment.

DISCUSSION

This is the first prospective, clinical study concerning CXCL12 in RA. The p-CXCL12 level was significantly elevated in 36 patients with long-standing RA compared to that in 50 healthy controls. This finding indicates that the p-CXCL12 level might serve as a diagnostic marker for RA. However, the specificity of the high p-CXCL12 level in RA should be clarified by comparison to its level in patients with OA, systemic lupus erythematosus, and chronic inflammatory bowel disease, etc.

Chemokines have generally been regarded as locally acting, paracrine or autocrine proteins, and several authors have reported a high local production of CXCL12 in RA joints, considerably higher than in OA and normal joints^{11,16,22,40,48}. We observed that the CXCL12 level was markedly increased in the circulation of patients with RA. Whether this represents a “spill-over” from the joints or hitherto unnoticed extraarticular production of this chemokine is not known. Simultaneous measurements of CXCL12 in both synovial biopsies and synovial fluid from affected joints and plasma could have elucidated this question. However, ethical and practical obstacles were a hindrance.

p-CXCL12 remained at a fixed high level among RA patients throughout the 28-week study period and was independent of any ACR disease activity variable, as well as response to MTX treatment. A constant and high p-CXCL12 level, uninfluenced by a decreasing disease activity and inflammatory activity over time, is in agreement with RA as a chronic, incurable condition, orchestrated by many cellular

Table 1. ACR disease activity variables in 36 patients with long-standing RA (> 6 mo) before starting (Week 0) and after 16 and 28 weeks of MTX therapy. Values are mean (range). Plasma CXCL12 data are given for comparison.

	Week 0	Week 16	Week 28
CRP, nmol/l	252	230	172
No. of swollen joints, maximum 40	6.4 (0–24)	4.0 (0–17)	3.7 (0–16)
No. of tender joints, maximum 42	10 (0–36)	6.7 (0–36)	7.1 (0–32)
HAQ score, 0–3	0.84 (0–2.38)	0.72 (0–2.25)	0.57 (0–1.38)
Physician’s global activity assessment, 5 point NRS	1.9 (0–4)	1.3 (0–3)	1.6 (0–3)
Patient’s global activity assessment, 10 point NRS	5.2 (0–9)	3.3 (0–8.5)	3.5 (1–8)
Patient’s referral of pain, 10 point NRS	2.8 (0–8)	1.9 (0–7)	2 (0–7.5)
Plasma CXCL12, mean (95% CI)	1854 (1709–1999)	1694 (1576–1812)	1783 (1643–1923)

NRS: numeric rating scale.

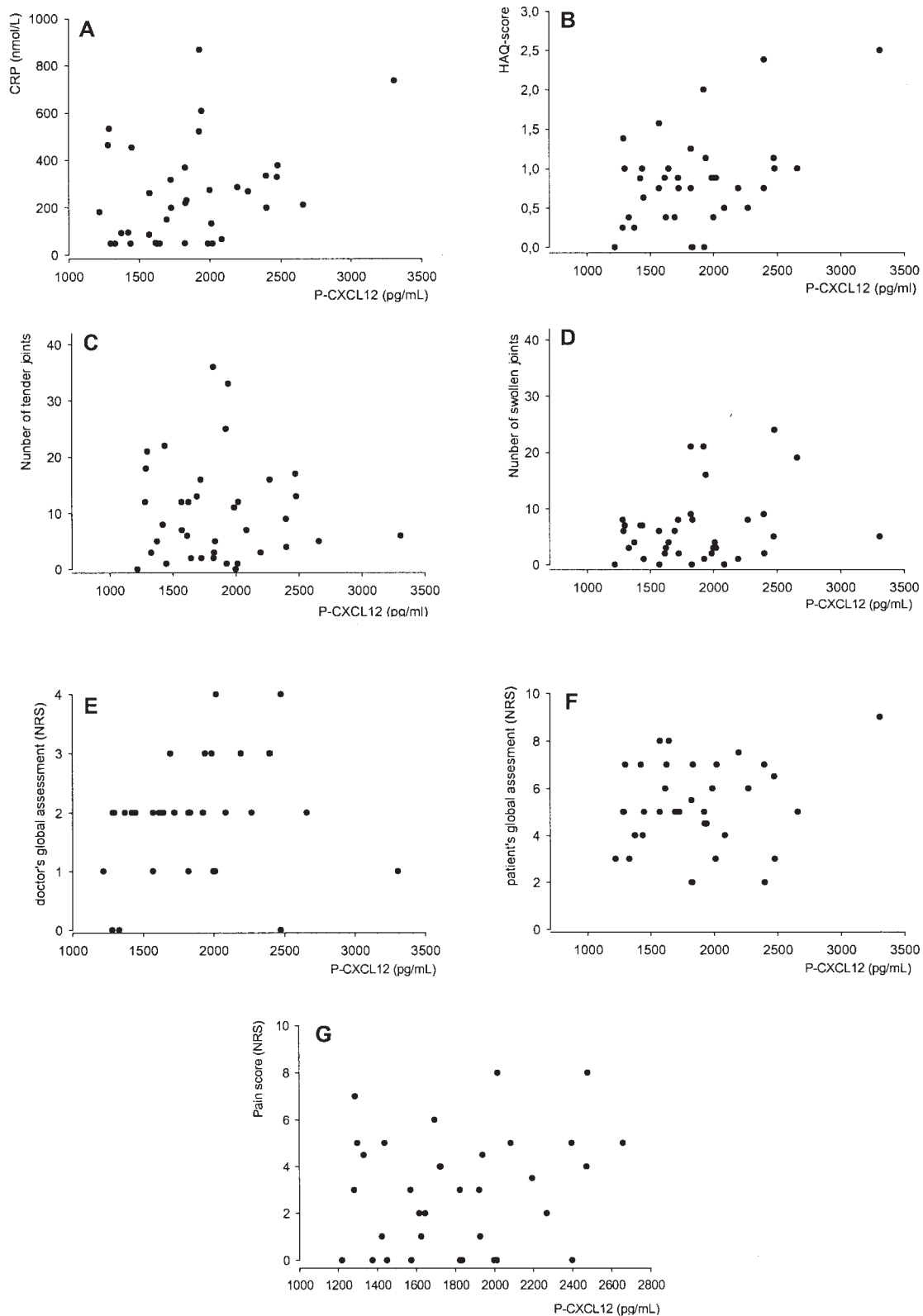


Figure 3. Correlation between p-CXCL12 and disease activity (ACR variables) in 36 patients with RA prior to start with MTX. A: C-reactive protein (CRP). B: Patient Health Assessment Questionnaire (HAQ). C: Number of tender joints. D: Number of swollen joints. E: Doctor's global assessment of RA activity. F: Patients' global assessment of RA activity, numeric rating scale (NRS). G: Patients' pain score. r = correlation coefficient. p = probability (Spearman's rank order). A: $r = 0.29$, $p = 0.081$. B: $r = 0.14$, $p = 0.419$. C: $r = 0.02$, $p = 0.912$. D: $r = 0.32$, $p = 0.055$. E: $r = 0.28$, $p = 0.097$. F: $r = 0.09$, $p = 0.615$. G: $r = 0.12$, $p = 0.52$.

and molecular proinflammatory activities, including production of CXCL12 from endothelial cells and FLS^{6,14}. Recent data showing elevated CXCL12 in RA synovial fluid⁴⁰ and the continuous expression of CXCL12 in RA synovial tissue, irrespective of an excellent treatment response to anti-TNF- α ⁴¹, are also in good agreement with our finding of a constantly elevated p-CXCL12 level over time, independent of response to MTX.

The lack of correlation between level of p-CXCL12 and any single ACR disease activity criterion, including C-reactive protein, indicates that CXCL12 is not "just another acute phase reactant." This view gains support by the absence of significant differences between the values of RA disease activity variables from the one-third of patients with the highest p-CXCL12 and one-third of patients with the lowest p-CXCL12.

Our validation of the CXCL12 ELISA showed only small inter- and intraassay variations and therefore the variation in our measurements cannot explain our findings.

In summary, this clinical, prospective study presents evidence of increased p-CXCL12 level in patients with RA. The p-CXCL12 level was constant over time and was not associated with RA disease activity variables (ACR) or response to MTX and oral steroid treatment.

ACKNOWLEDGMENT

The authors thank Lis Lund for excellent technical contribution and Dorit Sylvest for skilled secretarial assistance.

REFERENCES

1. Haringman JJ, Ludikhuizen J, Tak PP. Chemokines in joint disease: the key to inflammation? *Ann Rheum Dis* 2004;63:1186-94.
2. Luster AD. Chemokines — chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;338:436-45.
3. Murdoch C, Finn A. Chemokine receptors and their role in inflammation and infectious diseases. *Blood* 2000;95:3032-43.
4. Campbell JJ, Butcher EC. Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. *Curr Opin Immunol* 2000;12:336-41.
5. Campbell DJ, Kim CH, Butcher EC. Chemokines in the systemic organization of immunity. *Immunol Rev* 2003;195:58-71.
6. Buckley CD. Why do leucocytes accumulate within chronically inflamed joints? *Rheumatology Oxford* 2003;42:1433-44.
7. Schall TJ, Bacon KB. Chemokines, leukocyte trafficking, and inflammation. *Curr Opin Immunol* 1994;6:865-73.
8. Moser B, Loetscher P. Lymphocyte traffic control by chemokines. *Nat Immunol* 2001;2:123-8.
9. Mirshahi F, Pourtau J, Li H, et al. SDF-1 activity on microvascular endothelial cells: consequences on angiogenesis in vitro and in vivo models. *Thromb Res* 2000;99:587-94.
10. Salcedo R, Oppenheim JJ. Role of chemokines in angiogenesis: CXCL12/SDF-1 and CXCR4 interaction, a key regulator of endothelial cell responses. *Microcirculation* 2003;10:359-70.
11. Nanki T, Hayashida K, El Gabalawy HS, et al. Stromal cell-derived factor-1-CXC chemokine receptor 4 interactions play a central role in CD4+ T cell accumulation in rheumatoid arthritis synovium. *J Immunol* 2000;165:6590-8.
12. Bleul CC, Fuhlbrigge RC, Casasnovas JM, Aiuti A, Springer TA. A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). *J Exp Med* 1996;184:1101-9.
13. Bleul CC, Farzan M, Choe H, et al. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* 1996;382:829-33.
14. Nanki T, Lipsky PE. Cutting edge: stromal cell-derived factor-1 is a costimulator for CD4+ T cell activation. *J Immunol* 2000;164:5010-4.
15. Entschladen F, Gunzer M, Scheuffele CM, Niggemann B, Zanker KS. T lymphocytes and neutrophil granulocytes differ in regulatory signaling and migratory dynamics with regard to spontaneous locomotion and chemotaxis. *Cell Immunol* 2000;199:104-14.
16. Nanki T, Lipsky PE. Cytokine, activation marker, and chemokine receptor expression by individual CD4+ memory T cells in rheumatoid arthritis synovium. *Arthritis Res* 2000;2:415-23.
17. Salmon M, Scheel-Toellner D, Huissoon AP, et al. Inhibition of T cell apoptosis in the rheumatoid synovium. *J Clin Invest* 1997;99:439-46.
18. Suzuki Y, Rahman M, Mitsuya H. Diverse transcriptional response of CD4(+) T cells to stromal cell-derived factor (SDF)-1: cell survival promotion and priming effects of SDF-1 on CD4(+) T cells. *J Immunol* 2001;167:3064-73.
19. Pilling D, Akbar AN, Girdlestone J, et al. Interferon-beta mediates stromal cell rescue of T cells from apoptosis. *Eur J Immunol* 1999;29:1041-50.
20. Amft N. The role of CXCR4 in the inappropriate retention of T cells in the rheumatoid synovium. *EULAR conference 2000*; poster 126.
21. Buckley CD, Amft N, Bradfield PF, et al. Persistent induction of the chemokine receptor CXCR4 by TGF-beta 1 on synovial T cells contributes to their accumulation within the rheumatoid synovium. *J Immunol* 2000;165:3423-9.
22. Bradfield PF, Amft N, Vernon-Wilson E, et al. Rheumatoid fibroblast-like synoviocytes overexpress the chemokine stromal cell-derived factor 1 (CXCL12), which supports distinct patterns and rates of CD4+ and CD8+ T cell migration within synovial tissue. *Arthritis Rheum* 2003;48:2472-82.
23. Pap T, Muller-Ladner U, Gay RE, Gay S. Fibroblast biology. Role of synovial fibroblasts in the pathogenesis of rheumatoid arthritis. *Arthritis Res* 2000;2:361-7.
24. Buckley CD, Pilling D, Lord JM, Akbar AN, Scheel-Toellner D, Salmon M. Fibroblasts regulate the switch from acute resolving to chronic persistent inflammation. *Trends Immunol* 2001;22:199-204.
25. Pap T, Franz JK, Hummel KM, Jeisy E, Gay R, Gay S. Activation of synovial fibroblasts in rheumatoid arthritis: lack of expression of the tumour suppressor PTEN at sites of invasive growth and destruction. *Arthritis Res* 2000;2:59-64.
26. Pablos JL, Santiago B, Galindo M, et al. Synoviocyte-derived CXCL12 is displayed on endothelium and induces angiogenesis in rheumatoid arthritis. *J Immunol* 2003;170:2147-52.
27. Jackson JR, Seed MP, Kircher CH, Willoughby DA, Winkler JD. The codependence of angiogenesis and chronic inflammation. *FASEB J* 1997;11:457-65.
28. Nagasawa T. Role of chemokine SDF-1/PBSF and its receptor CXCR4 in blood vessel development. *Ann NY Acad Sci* 2001;947:112-5.
29. Murdoch C, Monk PN, Finn A. Functional expression of chemokine receptor CXCR4 on human epithelial cells. *Immunology* 1999;98:36-41.
30. Salvucci O, Yao L, Villalba S, Sajewicz A, Pittaluga S, Tosato G. Regulation of endothelial cell branching morphogenesis by endogenous chemokine stromal-derived factor-1. *Blood* 2002;99:2703-11.
31. Salcedo R, Wasserman K, Young HA, et al. Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: In vivo neovascularization induced by stromal-derived factor-1 alpha. *Am J Pathol*

- 1999;154:1125-35.
32. Nakamura H, Yoshino S, Ishiuchi N, Yokoyama M, Hiraishi K. Angiogenesis and angiogenic growth factors in the synovium in early rheumatoid arthritis [abstract]. *Arthritis Rheum* 1996;39 Suppl:S196.
 33. Walsh DA. Angiogenesis and arthritis. *Rheumatology Oxford* 1999;38:103-12.
 34. Borzi RM, Mazzetti I, Cattini L, Uguccioni M, Baggiolini M, Facchini A. Human chondrocytes express functional chemokine receptors and release matrix-degrading enzymes in response to C-X-C and C-C chemokines. *Arthritis Rheum* 2000;43:1734-41.
 35. Fearon U, Reece R, Smith J, Emery P, Veale DJ. Synovial cytokine and growth factor regulation of MMPs/TIMPs: implications for erosions and angiogenesis in early rheumatoid and psoriatic arthritis patients. *Ann NY Acad Sci* 1999;878:619-21.
 36. Masuko-Hongo K, Sato T, Nishioka K. Chemokines differentially induce matrix metalloproteinase-3 and prostaglandin E-2 in human articular chondrocytes 2. *Clin Exp Rheumatol* 2005;23:57-62.
 37. Yoshihara Y, Nakamura H, Obata K, et al. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis. *Ann Rheum Dis* 2000;59:455-61.
 38. Heesen M, Berman MA, Hopken UE, Gerard NP, Dorf ME. Alternate splicing of mouse fusin/CXC chemokine receptor-4: stromal cell-derived factor-1 alpha is a ligand for both CXC chemokine receptor-4 isoforms. *J Immunol* 1997;158:3561-4.
 39. Balabanian K, Lagane B, Infantino S, et al. The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. *J Biol Chem* 2005;280:35760-6.
 40. Kanbe K, Takagishi K, Chen Q. Stimulation of matrix metalloprotease 3 release from human chondrocytes by the interaction of stromal cell-derived factor 1 and CXC chemokine receptor 4. *Arthritis Rheum* 2002;46:130-7.
 41. van Oosterhout M, Levarht EW, Sont JK, Huizinga TW, Toes RE, van Laar JM. Clinical efficacy of infliximab plus methotrexate in DMARD naive and DMARD refractory rheumatoid arthritis is associated with decreased synovial expression of TNF alpha and IL18 but not CXCL12. *Ann Rheum Dis* 2005;64:537-43.
 42. Leaning J. War crimes and medical science. Declaration of Helsinki. *BMJ* 1996;313:1413-5.
 43. 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.
 44. Fuchs HA, Brooks RH, Callahan LF, Pincus T. A simplified twenty-eight-joint quantitative articular index in rheumatoid arthritis. *Arthritis Rheum* 1989;32:531-7.
 45. Wolfe F, Kleinheksel SM, Cathey MA, Hawley DJ, Spitz PW, Fries JF. The clinical value of the Stanford Health Assessment Questionnaire Functional Disability Index in patients with rheumatoid arthritis. *J Rheumatol* 1988;15:1480-8.
 46. 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.
 47. Felson DT, Anderson JJ, Boers M, et al. The American College of Rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. The Committee on Outcome Measures in Rheumatoid Arthritis Clinical Trials. *Arthritis Rheum* 1993;36:729-40.
 48. Burman A, Haworth O, Hardie DL, et al. A chemokine-dependent stromal induction mechanism for aberrant lymphocyte accumulation and compromised lymphatic return in rheumatoid arthritis. *J Immunol* 2005;174:1693-700.