# Plasma Monocyte Chemoattractant Protein 1 Is a Marker for Joint Inflammation in Rheumatoid Arthritis

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**ABSTRACT. Objective.** Monocyte chemoattractant protein 1 (MCP-1) level in plasma is described as a marker for joint inflammation in rheumatoid arthritis (RA).

*Methods.* MCP-1 in plasma and synovial fluid (SF) was quantified by ELISA in 36 RA patients with synovitis of the knee at Day 1 and 30. Disease activity was assessed by the swollen joint count, Ritchie Articular Index (RAI), global assessment, pain on visual analog scale, Health Assessment Questionnaire, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).

*Results.* By linear regression analysis plasma MCP-1 levels correlated significantly with the swollen joint count (Day 1: R = 0.47, p = 0.005; Day 30: R = 0.53, p < 0.001) and the RAI (Day 1: R = 0.37, p = 0.03; Day 30: R = 0.41, p = 0.01). The correlations of swollen joint count and RAI with ESR and CRP were significant only on Day 30 for the ESR (R = 0.40, p = 0.02). No association was found between plasma MCP-1 levels and the ESR/CRP levels. MCP-1 levels in plasma in RA patients were elevated compared to controls (p < 0.001) and MCP-1 levels in SF were higher than in plasma (p < 0.001). No correlation was found between SF MCP-1 levels and *in vitro* migration of mononuclear cells towards SF. MCP-1 appears to participate in the disease process in RA, and plasma MCP-1 may be useful in monitoring joint inflammation. (J Rheumatol 2001;28:41–6)

Key Indexing Terms:RHEUMATOID ARTHRITISMCP-1SYNOVITISMIGRATION

In rheumatoid arthritis (RA) swollen and tender joint counts are the clinical variables, while erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are the biochemical indicators preferred for monitoring inflammatory activity<sup>1-10</sup>. Of particular interest is the observation that swollen joint count is equally or even more strongly correlated with the development of erosions than ESR and CRP<sup>6,8,10</sup>. Thus there is a need for the development of a simple laboratory test that correlates to clinical joint inflammation and preferably also to erosive disease.

Monocyte chemoattractant protein 1 (MCP-1) is a monocyte, B and T lymphocyte chemoattractant *in vitro*<sup>11-13</sup>, as reviewed by Luster<sup>14</sup>. MCP-1 has been located to macrophages, endothelium, synovial fibroblasts, and chondrocytes in the inflamed joint from patients with RA<sup>15-18</sup>.

Taylor, *et al* have shown that tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) blockade is associated with a reduction in synovial membrane expression of MCP-1 positive cells as well as a

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reduction in plasma levels of MCP-1<sup>19</sup>. Methotrexate treatment of RA patients lowers the plasma level of MCP-1 and RANTES (regulated upon activation normal T cell expressed and secreted) in patients without radiological progression<sup>20</sup>.

These *in vitro* and *in vivo* observations led to the hypothesis that MCP-1 is important in the regulation of inflammation in RA. We observed that in RA, MCP-1 is increased in both plasma and synovial fluid (SF), with the highest concentration in the SF and a significant positive correlation between plasma levels of MCP-1 and the number of swollen joints as well as the Ritchie Articular Index (RAI). This indicates that plasma MCP-1 could be a new marker for joint inflammation.

# MATERIALS AND METHODS

Study patients. Inclusion criteria were: fulfillment of the 1987 American College of Rheumatology (ACR) criteria for  $RA^{21}$ , synovitis in one knee with indication for arthrocentesis, age between 18 and 75 years, and written acceptance of conditions for participation. Exclusion criteria were: intraarticular steroid and change in disease modifying antirheumatic drug (DMARD) in the inflamed knee within the preceding 3 months, other important illness, and pregnancy. RA patients were 27 women and 9 men followed over one month. Age varied from 31 to 75 years with a median of 60 years. Twenty-two were rheumatoid factor (RF) positive. Treatment was methotrexate in 11 patients, salazopyrin in 7, gold sodium thiomalate 2, penicillamine 8, auranofin 1, and hydroxychloroquine 1. Six patients were not taking DMARD. The median disease duration was 12 years, with a range from 4 months to 39 years. In 27 of the 36 patients Larsen score of the inflamed knee was median 2, mean 1.44 (range 0-3)<sup>9</sup>.

Disease activity of each patient was assessed by swollen joint count (maximum number 40), RAI, in which each joint was scored on a 4 point scale (0: no pain at pressure, 1: pain at pressure, 2: pain and wince at pressure, 3: pain and withdrawal at pressure)<sup>22</sup>, and physician global assessment of disease

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activity on a 5 point scale. The same physician carried out all clinical observations. The Stanford Health Assessment Questionaire (HAQ) for functional activity, visual analog (VAS) pain score for the body as a whole, and duration of morning stiffness (minimum to maximum improvement in or around joints, as in the ACR criteria).

ESR, CRP, hemoglobin, and RF (IgM-RF) were determined on Days 1 and 30. Clinical and biochemical characteristics of the RA patients are listed in Table 1.

MCP-1 was further quantified in plasma from 12 healthy persons recruited among staff members, 6 men and 6 women, median age 40 years (range 33–59).

The study was carried out at the Department of Rheumatology, Århus University Hospital, and was approved by the Ethics Committee of Århus County.

Laboratory investigations. Plasma and SF samples, CRP and ESR. All plasma samples were drawn in heparinized tubes between 8 and 11 AM. To each of the SF samples we added 50  $\mu$ l 10% w/v tetracemindinatrium per 10 ml after arthrocentesis. Plasma and SF samples were frozen in aliquots and stored at  $-80^{\circ}$ C for later analysis. ESR was determined according to Westergren; CRP was analyzed using a Behring Nephelometer (Behringwerke AG Diagnostica, Germany).

*MCP-1 ELISA*. The method for analysis of MCP-1 was based on an ELISA kit from R&D Systems, Abingdon, UK (Cat. no. DCP00). To determine the intraassay variation, one sample from each of 3 different patients was assayed 19 times on one plate, resulting in a coefficient of variation (CV) with a mean of 6.6% and a range of 6.2–7.1%. To determine the interassay precision one sample from each of 2 patients was assayed in triplicate in 3 different assays on the same day, resulting in a CV mean of 7.5%, range 5.6–9.4%. The day-to-day variation was determined by analyzing one plasma sample in triplicate in 13 assays on 6 different days, resulting in a CV = 6.2%.

The recovery of MCP-1 was tested by adding 2500 pg of recombinant human MCP-1 (R&D Systems) to 5 ml of 3 different plasma samples assayed in the same plate, compared to similar analysis of the same plasma sample without added recombinant MCP-1. This resulted in a mean recovery of 98.6% with a range of 86–113% in the 3 different plasma samples tested. Plasma samples (undiluted MCP-1 measured to 266 pg/ml and 230 pg/ml) diluted by 1:2, 1:4, 1:8, 1:16, and 1:32 resulted in a linear relation parallel to the standard curve.

In vitro migration of mononuclear cells towards SF. In vitro migration of mononuclear cells towards SF samples was carried out in the modified Boyden chamber, using DNA measurement by Hoechst 33258 (DQ201 fluorescent dye; Pharmacia Biotech, Stockholm, Sweden, Cat. no. B-2883) as quantification of migrated adherent cells. The technique has been described in detail<sup>23</sup>. Briefly, the peripheral blood mononuclear cells were isolated from heparinized venous blood from healthy persons immediately after sampling by flotation on Ficoll (Pharmacia). The cells were washed and resuspended in RPMI-1640 medium without L-glutamine (Pharmacia) in a concentration of 3 million/ml. The migration chambers (Neuro Probe Inc., Cabin John, MD, USA) had 10 wells in each unit with a migration area of 50.3 mm<sup>2</sup> per well and a bottom well volume of 150 µl. Polyvinyl-pyrolidone-free polycarbonate filters with a pore size of 5 µm were used (Nuchopore Corp., Pleasanton, CA, USA). SF diluted 1:5 in RPMI-1640 medium without L-glutamine was added to the bottom wells in triplicates for each experiment. The mononuclear cells (250 µl) were applied to the top wells. The chambers were incubated for 60 min at 37°C in humidified air containing 5% CO<sub>2</sub>. The filter was removed from the chamber. The top side was washed 3 times in Hanks' balanced with salt, scraped 3 times over a cell scraper (Neuro Probe Inc.), and dried at room temperature. The membrane area corresponding to the area of one well was manually cut out. The membrane was placed in a 250 µl TNE buffer (10 mM Tris-HCl, 1 mM HCl, 2 M NaCl), and to release the DNA from the cells, the samples were sonicated for 20 s. After centrifugation for 5 min at 8000 g, samples were added to 750 µl of the TNE buffer and 1 ml of the fluorochrome bisbenzimidazole solution Hoechst 33258 (1 µl/ml TNE buffer). A fluorescence detector (Perkin Elmer LS-5) determined the fluorescence with an excitation wavelength of 360 nm, and emission was recorded at 450 nm. The apparatus was equilibrated according to the manufacturer's instructions by the use of calf thymus DNA (Hoefer Pharmacia Biotech, Stockholm, Cat. No. 80622706). Cell standard plots for the corresponding DNA content of the migrating cells were performed each day. All fluorescence experiments were performed at room temperature. Assay variation including day-to-day variance and well-to-well variation has been described using SF samples from RA patients as chemoattractant<sup>23</sup>.

*Statistical analysis.* Linear regression analyses were used to assess the correlation between variables in the assessment of disease activity (the swollen joint count, the RAI, global assessment of disease activity by physician, VAS pain, and the HAQ score) and the biochemical variables (plasma MCP-1, ESR, CRP). In the statistical analysis of the VAS logit transformation was used. The determination coefficients, R, were used to explain the proportional variation due to the regression analysis. Significance was defined at the 5% level. Differences between groups were analyzed by t test. Significance was defined at the 5% level. All calculations were performed using Sigma Stat.

# RESULTS

In 36 patients with RA MCP-1 was detected in SF in the range 165 to 29,566 pg/ml, corresponding to a mean of 2362 pg/ml and median of 953 pg/ml. In plasma the range detected was 0-13,072 pg/ml, corresponding to a mean of 553 pg/ml and median of 303 pg/ml. All ELISA results from RA patients are listed in Table 1. In plasma from healthy controls (n = 12) the range detected was 101-234 pg/ml, corresponding to a mean of 158 pg/ml, median 152 pg/ml, and standard error of mean (SEM) 12.5 pg/ml.

The concentration of MCP-1 detected in SF was significantly higher than in the plasma from the same patient on the day of arthrocentesis (p < 0.001) (Table 1). No significant difference was found between plasma levels on the day of arthrocentesis (Day 1) and 30 days later (Day 30) (p = 0.5; see Table 2). The levels of MCP-1 in plasma from healthy controls were significantly lower than patients with RA (p < 0.001).

Results of physician clinical assessment of disease activity

*Table 1.* Disease activity variables and MCP-1 levels in plasma and synovial fluid (SF) for the 36 patients with RA. Plasma MCP-1 levels were significantly higher in SF compared to plasma (t test: p < 0.001).

	Day	Mean	Median	SEM
Number of swollen joints	1	20.1	23	1.40
-	30	19.6	19	1.43
Ritchie Articular Index	1	10.1	11	0.86
	30	10.9	12	1.05
Global assessment (MD)	1	2.1	2	0.18
	30	2.2	2	0.11
Pain on VAS, mm	1	26.1	23	4.14
	30	11.4	8	2.48
HAQ score	1	1.2	1.13	0.080
	30	1.1	1.00	0.094
CRP, mg/l	1	41	32	5.80
	30	44	36	6.45
ESR, mm/h	1	45	40	4.51
	30	46	46	4.71
RA plasma MCP-1, pg/ml	1	717	306	399
	30	390	291	78.1
RA SF, pg/ml	1	2362	953	931

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	Day	Plasma MCP-1		ESR		CRP	
		R	р	R	р	R	р
No. of swollen joints	1	0.47	0.005	0.22	0.20	0.04	0.81
	30	0.53	< 0.001	0.27	0.13	0.27	0.13
Ritchie Articular Index	1	0.37	0.03	0.26	0.13	0.18	0.32
	30	0.41	0.01	0.40	0.02	0.32	0.06
Global assessment (MD)	1	0.29	0.12	0.20	0.29	0.03	0.62
	30	0.21	0.22	0.17	0.35	0.41	0.02
Pain on VAS	1	0.18	0.35	0.15	0.44	0.10	0.62
	30	0.23	0.19	0.27	0.14	0.33	< 0.07
HAQ score	1	0.08	0.67	0.33	0.06	0.17	0.33
	30	0.08	0.87	0.31	0.09	0.23	0.21
CRP	1	0.03	0.87	0.32	0.07		
	30	0.21	0.25	0.64	< 0.001		
ESR	1	0.20	0.12				
	30	0.25	0.16	_		_	

Table 2. Results of linear regression analysis of correlations between plasma MCP-1, ESR, CRP, and variables of disease activity in the 36 patients with RA.

p < 0.05 was considered significant (in bold print).

(swollen joint count, RAI, global assessments), patient evaluation by HAQ score and VAS pain, CRP and ESR for Day 1 and Day 30 are given in Table 1 (values are mean, median, and SEM). No significant change was observed in any of the disease activity variables in Table 1 comparing Day 1 and 30 (number of swollen joints, p = 0.75; RAI, p = 0.55; global assessment, p = 0.57; HAQ, p = 0.50; VAS pain, p = 0.27; ESR, p = 0.87; and CRP, p = 0.97). Linear regression analyses of plasma MCP-1, ESR, and CRP to swollen joint count, RAI, global assessment of disease activity, VAS pain, and HAQ score are listed in Table 2. The association of plasma MCP-1, CRP, and ESR with the swollen joint count at Day 1 and Day 30 is shown in Figure 1. Regression lines are shown only for regression analysis showing significant correlations (p < 0.05).

Linear regression analysis showed significant positive correlations of plasma MCP-1 to swollen joint count (Day 1: R = 0.47, p = 0.005; Day 30: R = 0.53, p < 0.001) and RAI (Day 1: R = 0.37, p = 0.03; Day 30: R = 0.41, p = 0.01). MCP-1 level showed a stronger correlation with the swollen joint count than ESR and CRP.

No significant correlation was detected between plasma MCP-1 and ESR or CRP. Between ESR and CRP a positive significant correlation at Day 30 was present (Day 30: R = 0.64, p < 0.001). CRP levels were correlated significantly with the global assessment of disease activity on Day 30 (R = 0.41, p = 0.02). No correlation was found between the SF level of MCP-1 and disease activity variables in Table 2. *In vitro* migration of mononuclear cells towards the 36 SF samples was not correlated with SF MCP-1 levels (R = 0.06, p = 0.78). Larsen score did not correlate to plasma MCP-1 levels, SF MCP-1 levels, or *in vitro* migration (R = 0.08, p = 0.68; R = 0.25, p = 0.23; R = 0.01, p = 0.97).

# DISCUSSION

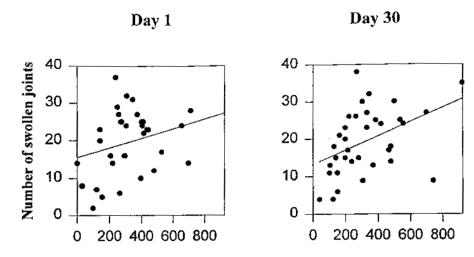
We investigated the usefulness of the chemokine MCP-1 as a biochemical marker of inflammatory activity in RA. The level of plasma MCP-1 in RA patients was found to be significantly elevated compared to the levels in normal healthy volunteers. The level of MCP-1 in plasma from healthy controls has recently been reported, with findings in the same range as our data<sup>24,25</sup>. As shown in Table 2, MCP-1 displayed a stronger correlation to the swollen joint count and the Ritchie Articular Index than did ESR and CRP. This is important since the number of swollen joints in particular correlates strongly with the risk of developing new erosions in the joints as evaluated by radiology<sup>5,6,8,10,26,27</sup>. Larsen score of the knee did not correlate to SF MCP-1 levels or *in vitro* migration of mononuclear cells towards SF.

Whether plasma MCP-1, or other chemokines, can be a prognostic marker for radiological progression of RA, as well as a marker of pharmacological response, needs to be evaluated in future studies.

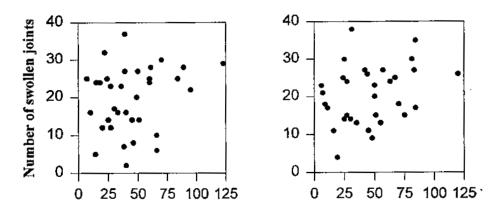
Treatment of RA patients with both methotrexate and TNF- $\alpha^{19,20}$  blockade reduces the level of plasma MCP-1. Taylor, *et al* also observed reduced numbers of CD3+ T cells, CD68+ macrophages, and MCP-1 positive cells in synovium of RA patients treated with one dose of TNF- $\alpha$  antibody blockade<sup>19</sup>.

Cytokines have also been evaluated as markers of disease activity. Interleukin 1ß (IL-1ß) has been reported to correlate with the RAI, pain scores, and ESR, although no correlation to the number of swollen joints has been reported<sup>28</sup>. IL-6 levels in plasma have been correlated with ESR and CRP in RA<sup>29</sup>. SF levels of IL-6 have been reported to correlate, although poorly, with the clinical inflammatory activity observed at a particular joint<sup>30</sup>. Several reports conclude that plasma IL-6 does not correlate with the overall synovial

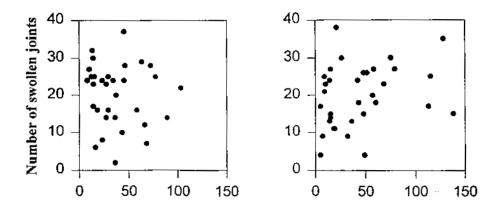
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Plasma Monocyte Chemoattractant Protein 1 pg/ml



Erythrocyte sedimentation rate mm/h



C-reactive protein, mg/l

*Figure 1.* The relation between the swollen joint count and plasma MCP-1, ESR, and CRP at Days 1 and 30. Results of significant correlations between the number of swollen joints and MCP-1 levels in plasma are shown by regression lines (Day 1, R = 0.47, p = 0.0005; Day 30, R = 0.53, p < 0.0001). No significant association was found between the acute phase response and the swollen joint count.

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inflammation evaluated by the swollen joint count<sup>30,31</sup>. Soluble IL-2 receptor (sIL-2r), soluble TNF receptor p55 (sTNFrp55), and soluble TNF receptor p75 (sTNFrp75) were shown to correlate with swollen joint counts, disease activity score, and CRP levels<sup>32</sup>. In this respect it is interesting that the only soluble cytokine receptors that correlate to swollen joint counts are those corresponding to cytokines thought to regulate chemokine expression in the arthritic joint or chemokine receptor expression in T lymphocytes generally<sup>19,33</sup>. *In vitro* it has been shown that MCP-1 preferentially attracts the Th1 subset of CD4+ T lymphocytes<sup>34</sup>, further indicating that MCP-1 may be important in the recruitment of predominantly the Th1 subset to the joint. Our data show the existence of a chemical gradient for MCP-1 towards the joint.

Several animal studies confirm the hypothesis of MCP-1 involvement in the recruitment of mononuclear cells to inflammatory lesions. In transgenic mice models<sup>35-37</sup>, where MCP-1 expression in vivo led to the accumulation of monocytes and lymphocytes locally at the site of expression, no local inflammatory activity was detected. Monocyte and lymphocyte migration could be blocked by neutralizing antibodies towards MCP-1 in one of these transgenic mouse models<sup>35</sup>. Further, the injection of human recombinant MCP-1 into the knee joints of rabbits led to the accumulation of mononuclear cells within the joint, resulting in histopathological changes resembling RA<sup>38,39</sup>. An antagonist of MCP-1 has been shown to inhibit arthritis in the MRL-lpr mouse model<sup>40</sup>. Survival and leukocyte traffic was shown to be MCP-1 dependent in MRL-lpr mice<sup>41</sup>. The reduction in joint swelling in the streptococcal wall, which induced arthritis after treatments of blocking antibodies against MCP-1, has also been reported<sup>42</sup>. Our results with in vitro migration of mononuclear cells towards 36 SF samples from RA patients showed no correlation between the number of migrated adherent cells and SF MCP-1 levels. Others have found that the lymphocyte in vitro chemoattractant activity of SF to be due to multiple factors<sup>43</sup>.

Based on these findings, a question to ask is why MCP-1 correlates to the degree of clinically assessed synovitis. MCP-1 can be localized to a large number of cell types present within the inflamed joint, including fibroblasts, T cells, macrophages, endothelial cells, chondrocytes, and subchondral bone marrow stromal cells<sup>15-18,44</sup>.

Others have found that the number of macrophages and the thickness of the lining layer in synovial membrane, evaluated by immunohistochemistry, correlates with radiographic progression<sup>45</sup>. Recently MCP-1 was tested *in vivo* in the rabbit cornea model and was found to be equally as potent as vascular endothelial growth factor (VEGF) in inducing new vessel formation<sup>46</sup>. VEGF in RA has been shown to correlate to CRP levels and not to Larsen score<sup>47</sup>. There is increasing evidence of neoangiogenesis in the synovium in RA<sup>48</sup>. Human endothelial cells have been shown to express mRNA for MCP-1 after *in vitro* stimulation by IL-1 and TNF but not by IL-6<sup>49,50</sup>. Further, smooth muscle cells express receptors for MCP-1<sup>51</sup>. Thus we suggest that the MCP-1 measured in plasma reflects the local inflammatory activity of several components of the arthritic joint in RA.

In summary, we found that in RA the MCP-1 level in plasma displayed a stronger correlation to the number of swollen and tender joints than the commonly used ESR and CRP. Taken together these data strengthen the notion that MCP-1 is a key molecule in the pathogenesis of RA and that plasma MCP-1 may be useful in monitoring synovial inflammatory activity.

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# REFERENCES

- 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.
- 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.
- Amos RS, Constable TJ, Crockson RA, et al. Rheumatoid arthritis: Relation of serum C-reactive protein and erythrocyte sedimentation rates to radiographic changes. BMJ 1977;1:195-7.
- Wolfe F, Sharp JT. Radiographic outcome of recent onset rheumatoid arthritis, a 19 year study of radiographic progression. Arthritis Rheum 1998;41:1571-82.
- Fex E, Eberhardt K, Saxne T. Tissue derived macromolecules and markers of inflammation in serum in early rheumatoid arthritis: Relationship to development of joint destruction in hands and feet. Br J Rheumatol 1997;36:1161-5.
- Prevoo MLL, van Riel PLCM, van 't Hof MA, et al. Validity and reliability of joint indices. A longitudinal study in patients with recent onset rheumatoid arthritis. Br J Rheumatol 1993;32:589-94.
- Plant MJ, Jones PW, Saklatvala J, Ollier WER, Dawes PT. Patterns of radiological progression in early rheumatoid arthritis: Results of an 8 year prospective study. J Rheumatol 1998;25:417-26.
- Matsuda Y, Yamanaka H, Higami K, Kashiwasaki S. Time lag between active joint inflammation and radiological progression in patients with early rheumatoid arthritis. J Rheumatol 1998;25:427-32.
- 9. Larsen A. The relation of radiographic changes to serum acute-phase proteins and rheumatoid factor in 200 patients with rheumatoid arthritis. Scand J Rheumatol 1988;17:123-9.
- van Leeuwen MA, van der Heijde DMFM, van Rijswijk MH, et al. Interrelationship of outcome measures and process variables in early rheumatoid arthritis. A comparison of radiologic damage, physical disability, joint counts and acute phase reactants. J Rheumatol 1994;21:425-9.
- Matsushima K, Larsen CG, DuBois GC, Oppenheim JJ. Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. J Exp Med 1989;169:1485-90.
- Taub DD, Proost P, Murphy WJ, et al. Monocyte chemotactic protein-1 (MCP-1), -2, and -3 are chemotactic for human T lymphocytes. J Clin Invest 1995;95:1370-6.
- Frade JM, Mellado M, del Real G, Gutierrez-Ramos J, Lind P, Martinez AC. Characterization of the CCR2 chemokine receptor: functional CCR2 receptor expression in B cells. J Immunol

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1997;159:5576-84.

- Luster AD. Chemokines chemotactic cytokines that mediate inflammation. N Engl J Med 1998;338:436-45.
- Koch AE, Kunkel SL, Harlow LA, et al. Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. J Clin Invest 1992;90:772-9.
- Harigai M, Hara M, Yoshimura T, Leonard EJ, Inoue K, Kashiwazaki S. Monocyte chemoattractant protein-1 in inflammatory joint diseases and its involvement in the cytokine network of rheumatoid synovium. Clin Immunol Immunopathol 1993;69:83-91.
- Hachicha M, Rathanaswami P, Schall TJ, McColl SR. Production of monocyte chemotactic protein-1 in human type B synoviocytes. Synergistic effect of tumor necrosis factor alpha and interferongamma. Arthritis Rheum 1993;36:26-34.
- Pulsatelli L, Dolzani P, Piacentini A, et al. Chemokine production by human chondrocytes. J Rheumatol 1999;26:1992-2001.
- Taylor PC, Peters MA, Paleolog E, et al. Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor alfa blockade in patients with rheumatoid arthritis. Arthritis Rheum 2000;43:38-47.
- Boiardi L, Macchioni P, Meliconi R, et al. Relationship between serum RANTES levels and radiological progression in rheumatoid arthritis patients treated with methotrexate. Clin Exp Rheumatol 1999;17:419-25.
- 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.
- 22. Ritchie DM, Boyle JA, McInnes JM, et al. Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. Q J Med 1968;37:393-406.
- Ellingsen T, Buus A, Møller B, Stengaard-Pedersen K. *In vitro* migration of mononuclear cells towards synovial fluid and plasma from rheumatoid arthritis patients correlate to RANTES synovial fluid levels and to clinical pain parameters. Scand J Rheumatol 2000;29:216-21.
- Ida N, Sakurai S, Kawano G. Detection of monocyte chemotactic and activating factor in normal blood and urine using a sensitive ELISA. Cytokine 1994;6:32-9.
- Inadera H, Egashira K, Takemoto M, Ouchi Y, Matsushima K. Increase in circulating levels of monocyte chemoattractant protein 1 with aging. J Interferon Cytokine Res 1999;19:1179-82.
- Ingeman Nielsen M, Halskov O, Hansen TM, Halberg P, Stage P, Lorenzen I. Clinical synovitis and radiological lesions in rheumatoid arthritis. A prospective study of 25 patients during treatment with remission-inducing drugs. Scand J Rheumatol 1983;12:237-40.
- Mottonen T. Prediction of erosiveness and rate of development of new erosions in early rheumatoid arthritis. Ann Rheum Dis 1988; 47:648-53.
- Eastgate JA, Symons JA, Wood NC, Grinlinton FM, di Giovine F, Duff GW. Correlation of plasma interleukin 1 levels with disease activity in rheumatoid arthritis. Lancet 1988;2:706-9.
- Cohick CB, Furst D, Quagliata S, et al. Analysis of elevated serum interleukin-6 levels in rheumatoid arthritis: Correlations with erythrocyte sedimentation rate or C-reactive protein. J Lab Clin Med 1994;123:721-7.
- Holt I, Cooper RG, Denton J, Meager A, Hopkins SJ. Cytokine interrelationships and their association with disease activity in arthritis. Br J Rheumatol 1999;31:725-33.
- 31. van Leeuwen MA, Westra J, Limburg PC, van Riel PL, van Rijswijk MH. Interleukin-6 in relation to other proinflammatory cytokines, chemotactic activity and neutrophil activation in rheumatoid synovial fluid. Ann Rheum Dis 1995;54:33-8.
- 32. Barrera P, Haagsma CJ, Boerbooms AM, et al. Effect of methotrexate alone or in combination with sulphasalazine on the production and circulating concentrations of cytokines and their antagonists.

Longitudinal evaluation in patients with rheumatoid arthritis. Br J Rheumatol 1995;34:747-55.

- Loetscher P, Seitz M, Baggiolini M, Moser B. Interleukin-2 regulates CC chemokine receptor expression and chemotactic responsiveness in T lymphocytes. J Exp Med 1996;184:569-77.
- Bonecchi R, Bianchi G, Bordignon PP, et al. Differential expression of chemokine receptors and chemotactive responsiveness of Type 1 T helper cells (Th1) and Th2. J Exp Med 1998;187:129-34.
- Gunn MD, Nelken NA, Liao X, Williams LT. Monocyte chemoattractant protein-1 is sufficient for the chemotaxis of monocytes and lymphocytes in transgenic mice but requires an additional stimulus for inflammatory activation. J Immunol 1997;158:376-83.
- Fuentes ME, Durham SK, Swerdel MR, et al. Controlled recruitment of monocytes and macrophages to specific organs through transgenic expression of monocyte chemoattractant protein-1. J Immunol 1995;155:5769-76.
- Grewal IS, Rutledge BJ, Fiorillo JA, et al. Transgenic monocyte chemoattractant protein-1 in pancreatic islets produces monocyte-rich insulitis without diabetes: abrogation by a second transgene expressing systemic MCP-1. J Immunol 1997;159:401-8.
- Akahoshi T, Wada C, Endo H, et al. Expression of monocyte chemotactic and activating factor in rheumatoid arthritis. Regulation of its production in synovial cells by interleukin-1 and tumor necrosis factor. Arthritis Rheum 1993;36:762-71.
- Nishimura A, Akahoshi T, Ohtaka F, et al. Experimental arthritis induced by continuous infusion of MCP-1 in rabbit knee joint [abstract]. Arthritis Rheum 1998;41 Suppl:391.
- Gong JH, Ratkay LG, Waterfield JD, Clark Lewis I. An antagonist of monocyte chemoattractant protein 1 inhibits arthritis in the MRL-lpr mouse model. J Exp Med 1997;186:131-7.
- Tesch GH, Maifert S, Schwarting A, Rollins BJ, Kelley VR. Monocyte chemoattractant protein 1-dependent leukocytic infiltrates are responsible for autoimmune disease in mrl-fas lpr mice. J Exp Med 1999;190:1813-24.
- Schimmer RC, Schrier DJ, Flory CM, et al. Streptococcal wallinduced arthritis: requirements for IL-4, IL-10, IFN-gamma, and monocyte chemoattractant protein 1. J Immunol 1998;160:1466-71.
- al Mughales J, Blyth TH, Hunter JA, Wilkinson PC. The chemoattractant activity of rheumatoid synovial fluid for human lymphocytes is due to multiple cytokines. Clin Exp Immunol 1996;106:230-6.
- 44. Lisignoli G, Toneguzzi S, Pozzi C, et al. Chemokine expression by subchondral bone marrow stromal cells isolated from osteoarthritis and rheumatoid arthritis patients. Clin Exp Immunol 1999;116:371-8.
- Mulherin D, Fitzgerald O, Bresnihan B. Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. Arthritis Rheum 1996;39:115-24.
- Goede V, Brogelli L, Ziche M, Augustin HG. Induction of inflammatory angiogenesis by monocyte chemoattractant protein 1. Int J Cancer 1999;82:765-70.
- Harada M, Mitsuyama K, Yoshida H, et al. Vascular endothelial growth factor in patients with rheumatoid arthritis. Scand J Rheumatol 1998;27:377-80.
- 48. Walsh DA. Angiogenesis and arthritis. Rheumatology 1999;38:103-12.
- 49. Sica A, Wang JM, Colotta F, et al. Monocyte chemotactic and activating factor gene expression induced in endothelial cells by IL-1 and tumor necrosis factor. J Immunol 1999;144:3034-8.
- Rollins BJ, Yoshimura T, Leonard EJ, Pober JS. Cytokine-activated human endothelial cells synthesize and secrete a monocyte chemoattractant, MCP-1/JE. Am J Pathol 1990;136:1229-33.
- Hayes IM, Jordan NJ, Towers S, et al. Human vascular smooth muscle cells express receptors for CC chemokines. Arterioscler Thromb Vasc Biol 1998;18:397-403.

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