CONCISE REPORT

Up regulation of monocyte chemoattractant protein-1 expression in anti-citrulline antibody and immunoglobulin M rheumatoid factor positive subjects precedes onset of inflammatory response and development of overt rheumatoid arthritis

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Objective: To analyse the inflammatory mediators, including monocyte chemoattractant protein-1 (MCP-1), in blood samples donated years before onset of rheumatoid arthritis. Previously, it has been shown in these individuals that antibodies against cyclic citrullinated peptide (anti-CCP) detectable years before the onset of symptoms have a high predictive value for development of rheumatoid arthritis.

Methods: A nested case-control study was performed: patients with rheumatoid arthritis were identified from among blood donors antedating onset by a median of three years (pre-patients, $n=92$); four matched controls were selected randomly for each pre-patient. Plasma were analysed for secretory phospholipase A2 (sPLA2) and MCP-1 using ELISA, for high-sensitivity C reactive protein (hsCRP) using the chemiluminescence method and for interleukin-6 using a sensitive bioassay.

Results: When the results were stratified for the presence of anti-CCP antibodies and immunoglobulin M-rheumatoid factor (IgM-RF), only MCP-1 levels were found to be significantly raised in patients with anti-CCP and IgM-RF compared with controls.

Conclusion: Levels of MCP-1 were significantly increased in the plasma of patients having anti-CCP antibodies or IgM-RF and who later developed rheumatoid arthritis. These findings indicate up regulation of chemotactic activity for leucocytes before the development of rheumatoid arthritis.

We have previously shown that anti-cyclic citrullinated (anti-CCP) antibodies and rheumatoid factors predate the onset of rheumatoid arthritis by several years.¹ The presence of anti-CCP antibodies predicted the development of rheumatoid arthritis,¹ the relative risk for which was further increased with carriage of the HLA-DRB1*0404/0401 and particularly the PTPN22 T1858C T variant.^{2,3}

Monocyte chemotactic protein-1 (MCP-1) is a chemokine expressed in the synovium in patients with rheumatoid arthritis⁴ and associated with leucocyte migration—for example, into the joint. It has been shown to stimulate T cells and interleukin (IL)6 and IL8 production by synovial fibroblasts. Soluble secretory phospholipase A2 (sPLA2) is involved in a variety of processes,⁵ with increased levels being found in inflammatory sites such as the synovium in patients with rheumatoid arthritis. Cytokines such as IL6, tumour necrosis factor and IL1 β regulate circulating levels of sPLA2 and C reactive protein (CRP) by affecting their synthesis and secretion

from various cell types.^{5,6} Reports regarding CRP and sPLA2 in people before onset of rheumatoid arthritis are contradictory.^{7–10}

The aim of the study was to analyse whether inflammatory mediators were increased in parallel with autoantibody production. In this study, inflammatory mediators including sPLA2, CRP, IL6 and MCP-1 were analysed in plasma from people who had donated blood to the Medical Biobank of northern Sweden (Umeå, Sweden) years before the onset of rheumatoid arthritis and in samples collected when diagnosed at an early arthritis clinic. The pre-patient data were stratified for the presence of anti-CCP antibodies or rheumatoid factor isotypes in statistical analyses.

MATERIALS AND METHODS

The register of patients with early rheumatoid arthritis (duration of <1 year) fulfilling the classification criteria for rheumatoid arthritis¹¹ at the Department of Rheumatology, Umeå, Sweden and with a known date of onset of symptoms, was co-analysed with the register of patients in the Medical Biobank of northern Sweden, as presented previously.¹ Fifty nine patients who later developed rheumatoid arthritis (referred to as pre-patients) were identified as blood donors. A further 33 donors were identified at a subsequent re-analysis of the registers. Consequently, a total of 92 pre-patients were identified, and for every sample four controls, matched for sex and age at the time of blood sampling, were randomly selected from the same subcohort. Due to other ongoing projects and occasional lack of samples, plasma was not available from all identified pre-patients. High-sensitivity CRP (hsCRP) and sPLA2 were analysed in the first cohort of identified pre-patients ($n=58$ and $n=54$, respectively) and controls ($n=231$ and $n=211$, respectively). MCP-1 and IL6 were analysed in the expanded group of pre-patients (84 and 74 pre-patient samples, and 323 and 292 controls, respectively). Serum samples from a maternity cohort ($n=24$)¹ were not included in any analysis of this study as CRP has been proposed as a serological marker for pre-term delivery.¹² The mean age (range) of all pre-patients when sampled was 53.3 (29.9–68.4) years. The median time of sampling before onset of symptoms was 3 years (interquartile range (IQR) 1.1–5.3), while the mean age at onset of symptoms was 56.7 years (range 34–73). The median time from onset of symptoms until diagnosis of early rheumatoid arthritis (≥ 4

Abbreviations: CCP, cyclic citrullinated peptide; CRP, C reactive protein; hsCRP, high-sensitivity C reactive protein; IgM-RF, immunoglobulin M-rheumatoid factor; MCP, monocyte chemoattractant protein; sPL, secretory phospholipase

American College of Rheumatology criteria fulfilled) was 7 months (IQR 5–9).

Plasma samples were analysed for sPLA2 and MCP-1 using ELISA (Cayman, Ann Arbor, Michigan, USA and HyCult Biotechnology, Uden, The Netherlands, respectively). The hsCRP was measured using the IMMULITE assay, a two-site chemiluminescent enzyme immunometric assay with one monoclonal and one polyclonal anti-CRP antibody with a detection limit specified by the manufacturer of 0.1 mg/l (Diagnostic Products, Los Angeles, California, USA). A 1:100 manual dilution provided a measurable range of 0.1–500 mg/l, and the interassay coefficient of variation was <6%. The level of IL6 was determined by the proliferation assay of the IL6-dependent cell line, B13.29. The amount of IL6 was calculated using dilutions of recombinant human IL6 (Genzyme, Cambridge, Massachusetts, USA) as previously described.¹³

Anti-CCP antibodies and rheumatoid factors of immunoglobulin M, immunoglobulin G and immunoglobulin A isotypes were measured using ELISA as described previously.¹ The PTPN22 1858C/T polymorphisms and HLA-DRB1 genotyping were determined as described previously.^{2, 3}

The χ^2 test was used for testing categorical data and continuous data were compared using non-parametric methods. All p values refer to two-sided tests and a p value ≤ 0.05 was considered significant. The calculations were performed using the SPSS package (SPSS for Mac 11.0.4).

RESULTS

The levels of MCP-1 were considerably increased in patients positive for anti-CCP antibodies before disease onset compared with controls (table 1).

The levels of MCP-1 were also significantly increased in patients positive for IgM-RF (1.34 (0.96–1.58) ng/ml) compared with IgM-RF negative (0.84 (0.64–1.30) ng/ml) pre-patients before disease onset ($p < 0.05$) and compared with controls ($p < 0.05$). There was no relationship between the level of MCP-1 and the time preceding disease onset. Among the pre-patients, the frequency of anti-CCP antibodies was significantly increased to 37.6%, IgM-RF to 22.2%, IgA-RF to 38.9% and IgG-RF to 16.7%. The presence of anti-CCP antibodies in pre-patients correlated significantly with the presence of IgM-RF (Fisher's exact test, $p < 0.05$) and IgA-RF ($\chi^2 = 5.61$, $p < 0.05$) but not with IgG-RF.

There was no significant increase in the levels of hsCRP, sPLA2 or IL6 in patients before disease onset irrespective of the presence of anti-CCP antibodies or of rheumatoid factors of any isotypes. The presence of HLA-SE or the PTPN22 1858T variant did not affect the levels of any of the measured factors.

In patients with early rheumatoid arthritis, the levels of MCP-1 and hsCRP were considerably increased compared with controls (table 1); none of the levels was related to the presence of anti-CCP antibodies or IgM-RF.

DISCUSSION

In the present study, analysis of samples from patients collected before onset of symptoms of joint disease showed increased levels of MCP-1 in anti-CCP and IgM-RF positive patients. Increased levels of MCP-1 have been shown to be present in the synovial fluid in patients with rheumatoid arthritis.¹⁴ Synovial fibroblasts from patients with rheumatoid arthritis produce MCP-1 in response to proinflammatory cytokines and interferon- γ ; also, articular chondrocytes secrete MCP-1.¹⁵ Our finding of increased levels of MCP-1 suggests that the disease process probably has started at a subclinical level. This is supported by our findings of an increased Larsen score in anti-CCP antibody positive patients when they present at an early arthritis clinic compared with those without these antibodies

Table 1 Levels of high-sensitivity C reactive protein, monocyte chemoattractant protein 1, secretory phospholipase A2 and interleukin-6 in pre-patients stratified for the presence of anti-cyclic citrullinated peptide antibodies, in individuals before onset of symptoms (pre-patients) and in patients with early rheumatoid arthritis and in controls

	Pre-patients		Patients with early rheumatoid arthritis		Controls
	Anti-CCP+	Anti-CCP–			
hsCRP (mg/l)	1.54 (0.64–2.83) (n = 22)	1.55 (0.94–2.92) (n = 36)	6.87 (2.82–22.60)* (n = 67)		1.43 (0.61–3.04) (n = 231)
MCP-1 (ng/ml)	1.05 (0.62–1.34)** (n = 32)	0.80 (0.54–1.29) (n = 52)	1.32 (0.84–1.80)* (n = 92)		0.71 (0.53–1.07) (n = 321)
sPLA2 (μ g/ml)	2.61 (2.06–3.67) (n = 20)	2.61 (2.20–3.48) (n = 34)	nt		2.63 (1.82–3.65) (n = 211)
IL-6 (pg/ml)	40 (31.5–51.5) (n = 24)	41 (32.0–51.5) (n = 50)	nt		44 (33.0–55.0) (n = 292)

CCP, cyclic citrullinated peptide; hsCRP, high-sensitivity C reactive protein; MCP, monocyte chemoattractant protein; sPL, secretory phospholipase; nt, not tested.

Data are presented as median (interquartile range).

* $p < 0.05$, ** $p < 0.001$.

preceding disease onset.¹⁶ Anti-CCP antibodies have been suggested to be involved in causing rheumatoid arthritis rather than being a consequence of existing disease. Our data are in line with this hypothesis. Another possibility is that release of chemokines such as MCP-1 could precede, as part of the innate immunity, the induction of antibody production (eg, anti-CCP antibodies), representing a somewhat later occurring acquired immune response.

There were no differences in MCP-1 levels in patients with early rheumatoid arthritis when they were stratified for the presence of anti-CCP antibodies. Nor were there any differences in other measurements of inflammatory activity (eg, erythrocyte sedimentation rate, swollen or tender joint count, disease activity score of 28) at baseline.¹⁶ Similar results have been presented by others.¹⁷ However, in a number of studies, the presence of anti-CCP antibodies at baseline has predicted a more aggressive disease with unfavourable outcome.^{16, 17}

None of the other acute-phase reactants assayed, —that is, hsCRP, sPLA2 or IL6—showed increased levels irrespective of the presence of autoantibodies (anti-CCP or rheumatoid factors). The results for hsCRP are consistent with those of Aho *et al*,⁷ who did not observe increased CRP levels in sera from 124 patients with a median follow-up time of 5–10 years. Conversely, there are two reports of increased levels of hsCRP.^{8, 10} In one study, 4 of 18 pre-rheumatoid arthritis cases had increased levels preceding disease onset compared with 4 of 72 controls ($p = 0.048$).⁸ In another, larger study involving several samples from each individual predating disease onset, the levels of hsCRP and sPLA2 were considerably increased.^{9, 10} Levels of both hsCRP and sPLA2 increased closer to the onset of disease symptoms. Since Nielen *et al*^{9, 10} had several samples for each patient, they attempted to analyse the sequence of increasing inflammation and increased autoantibody concentrations but were unable to identify any particular order. We were not able to confirm a time dependency for the levels, possibly due to the relatively low number of samples which could not overcome the inter-individual variability. Furthermore, the low number of samples in our study is a limitation for detection of differences of the measured

variables. However, the increased level of MCP-1 is in line with the hypothesis of the attraction of leucocytes to the joints or other lymph organs as an early event in the inflammatory process—that is preceding the production of the other factors we have measured.

In summary, levels of MCP-1 were considerably increased in anti-CCP and in IgM-RF positive patients before onset of symptoms of a joint disease. Thus, up regulation of chemotactic activity would suggest that leucocyte migration is an early process in the development of inflammation in rheumatoid arthritis.

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