Microheterogeneity of α_1 acid glycoprotein in rheumatoid arthritis: dependent on disease duration?

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Abstract

The microheterogeneity of α_1 acid glycoprotein (AGP) was studied using affinity immunoelectrophoresis with concanavalin A (Con A) in serum samples of 43 patients with early rheumatoid arthritis (RA) without clinical features of intercurrent infection. The results were expressed as reactivity coefficients. Disease activity was measured by clinical (Lansbury's joint index, Mallya-Mace activity score) and laboratory (erythrocyte sedimentation rate, levels of soluble interleukin-2 receptor, C reactive protein, and AGP) indices. In contrast with previous reports, suggesting a decrease in AGP-Con A reactivity in patients with RA, high values of AGP reactivity coefficients were found in patients with disease of short duration, which were similar to those found in patients with acute bacterial infections. Conversely, normal or decreased values of AGP reactivity coefficients were found in patients with disease of longer duration. Regression analysis showed a significant relation between AGP reactivity coefficients and disease duration (multiplicative model). No other indices examined were significantly related to disease duration.

These results, taken together with previous findings suggesting that cytokines control the glycosylation of acute phase proteins, indicate that differences in the microheterogeneity of AGP in early and longstanding RA reflect differences in cytokine action at different stages of the disease.

result, only biantennary structures can react with Con A,¹² and the total degree of reactivity depends on the number of these oligosaccharide chains coupled to the polypeptide chain.² Thus diverse glycoforms of AGP have different electrophoretic mobility in agarose gel containing Con A.⁶

Several studies have shown significantly increased AGP-Con A reactivity in patients with various acute inflammatory diseases and in patients with rheumatoid arthritis (RA) accompanied by intercurrent bacterial infections.^{5 9 11} On the other hand, decreased reactivity of AGP with Con A was found in RA and other chronic inflammatory conditions.^{3 5 7 8 10}

In contrast with the above findings, we found an increased AGP-Con A reactivity in patients with early RA. This observation prompted us to investigate the microheterogeneity of AGP in patients with early RA (disease duration less than 18 months) in relation to disease duration and activity.

Patients and methods

Fifty outpatients with recent onset arthritis were studied. Time of onset of the synovitis was assessed in each case according to the anamnestic data (onset of morning stiffness, joint pain, and swelling, followed by similar symptoms over at least six weeks). Patients with evidence of concomitant infections were not included in the study. All patients were examined physically and their disease activity assessed with a modified Mallya-Mace multivariate scale.¹³

Blood samples were taken and tested for C reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor, and antinuclear antibodies by standard techniques. Additionally, three serum samples for each patient were stored frozen at -20°C . To confirm the diagnosis patients were examined one year after the initial visit. Most patients ($n=43$) were diagnosed as having RA, according to the revised criteria of the American College of Rheumatology.¹⁴ There were 33 women (mean age 50 years (range 18–78), mean disease duration at the initial visit eight months (range 2–17)) and 10 men (mean age 49 years (range 33–61), mean disease duration at the initial visit seven months (range 2–16)). Twenty nine patients were seropositive, 14 seronegative. Seven patients (one who developed systemic sclerosis, one with osteoarthritis, two patients in complete remission, and three patients who could not be re-examined) were excluded from the study.

Analysis of the stored serum samples was

(*Ann Rheum Dis* 1993; 52: 138–141)

Most acute phase proteins are N-glycosylated—that is, they have oligosaccharides covalently attached to Asn through an N-glycosidic linkage. For instance, α_1 acid glycoprotein (AGP) has five complex-type, mannose-containing heteroglycan side chains bound to a single polypeptide chain.¹ Each of these oligosaccharides contains a core pentasaccharide, enlarged by the addition of two, three, or four outer chains (bi-, tri-, or tetra-antennary structures).^{2 3} The striking diversity of these heteroglycans (many structures may be found at the same glycosylation site) is usually referred to as microheterogeneity.³

To analyse the microheterogeneity of acute phase proteins crossed affinity immunoelectrophoresis with the lectin concanavalin A (Con A) has been used.^{4–11} For binding to Con A at least two interacting α linked 2-o-substituted mannose molecules with unmodified C-3, C-4, and C-6 hydroxyl groups are required. As a

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Accepted for publication 15 September 1992

carried out within six months after the initial visit.

Microheterogeneity of AGP was studied by crossed affinity immunoelectrophoresis with Con A.⁴ Concanavalin A (Sigma type 4, C-2010 Sigma, St Louis, USA) in a concentration of 40 μmol/l was incorporated into the first dimension

Table 1 Lansbury's joint index, Mallya-Mace activity score, erythrocyte sedimentation rate (ESR), C reactive protein (CRP), soluble interleukin-2 receptor (sIL-2R), α₁ acid glycoprotein (AGP), AGP reactivity coefficients (AGP-RC) in 43 patients with rheumatoid arthritis

	Mean	Standard deviation	Minimum	Maximum
Lansbury's joint index	40.73	30.18	0	124
Mallya-Mace activity score	2.38	0.53	1.3	3.5
ESR (mm/h) (normal <10)	48.95	29.93	5	120
CRP (mg/l) (normal <6)	20.47	16.65	0	80
sIL-2R (IU/ml) (normal <200)	1292.79	1282.07	0	5200
AGP (g/l) (normal 0.55-1.40)	1.76	0.70	0.75	3.47
AGP-RC (normal 1.28 (SD 0.25))	1.34	0.39	0.74	2.63

Regression curve (multiplicative model: $Y=1.236 \times -0.186$) of α₁ acid glycoprotein on disease duration (continuous line). The two dashed lines closest to the solid line indicate the limits of confidence ($p<0.05$). The two dashed lines farthest from the regression curve represent the limits of prediction ($p<0.05$).

gel, and first dimension electrophoresis was carried out for 1 h at 10 V/cm. Gel strips were then transferred onto the second dimension plate and two gels adjacent to the first dimension gel were poured; one contained antibodies to AGP (Dakopatts A 011, lot 039 B) and the other 7.5% methyl-α-D-mannopyranoside to solubilise the Con A-glycoprotein complexes. After second dimension electrophoresis (16-18 h, 1.5 V/cm) the gel was washed, dried, and stained with Coomassie brilliant blue R-250 (Sigma).

The presence of three to four microheterogeneous forms of AGP was established: variant 0 (non-reactive with Con A), variant 1 (weakly reactive), variant 2 (reactive), and variant 3 (strongly reactive). The area under each peak was determined by planimetry and the AGP reactivity coefficient was calculated by dividing the sum of the variants reactive with Con A by the variants not reactive with Con A. The AGP concentration was determined by electro-immunoassay. Commercial enzyme linked immunosorbent assay (ELISA) kits (Cell-free, T-cell Sciences Inc, Cambridge, MA, USA) were used for determination of soluble interleukin-2 receptor (sIL-2R). All samples were examined in duplicate.

STATISTICS
The results were analysed by descriptive statistics, Mann-Whitney U test, Spearman rank correlation, and analyses of variance and regression using linear, reciprocal, multiplicative, and exponential models.

Results
Table 1 shows the mean values of Lansbury's joint index, Mallya-Mace activity score, ESR, CRP, sIL-2R, AGP and AGP reactivity coefficients.
The highest AGP reactivity coefficients (2.63, 2.39, 2.16, 1.85) were found in the patients with the shortest disease duration, whereas normal or low AGP reactivity coefficients were found in patients with longer disease duration. (Normal mean value 1.28 (SD 0.25).) Statistical analysis of these findings showed a significant inverse correlation between disease duration and AGP reactivity coefficient ($r=-0.4369$, $p<0.005$). There was no significant correlation between disease duration and any other variable investigated.

Table 2 Spearman rank correlations between Lansbury's joint index, Mallya-Mace activity score, erythrocyte sedimentation rate (ESR), C reactive protein (CRP), soluble interleukin-2 receptor (sIL-2R), α₁ acid glycoprotein (AGP), AGP reactivity coefficients (AGP-RC) and duration of disease. Results are shown as correlation coefficient (significance level)

	Joint index	Activity	ESR	CRP	sIL-2R	AGP	AGP-RC
Activity	0.7405 (0.0000)						
ESR	0.6148 (0.0001)	0.6255 (0.0001)					
CRP	0.5886 (0.0001)	0.5818 (0.0002)	0.7042 (0.0000)				
sIL-2R	0.2019 (0.1907)	0.0099 (0.9486)	0.1881 (0.2228)	0.1865 (0.2268)			
AGP	0.3429 (0.0263)	0.1893 (0.2200)	0.4018 (0.0092)	0.4436 (0.0040)	0.2780 (0.0716)		
AGP-RC	-0.2388 (0.1217)	-0.3124 (0.0429)	-0.4974 (0.0013)	-0.2309 (0.1346)	0.0091 (0.9529)	-0.0565 (0.7141)	
Duration	-0.1980 (0.1994)	-0.1825 (0.2369)	-0.0441 (0.7749)	-0.1331 (0.3884)	0.2589 (0.0934)	0.1040 (0.5004)	-0.4369 (0.0046)

Analysis of regression of AGP reactivity coefficients in relation to disease duration showed the multiplicative model to be the most appropriate for fitting the data ($p < 0.005$, $R^2 = 20.65$, $r = -0.4545$) (figure).

Regression analysis of sIL-2R concentration in relation to disease duration suggested an exponential relation between these two variables, though significance was not reached ($p = 0.0768$, $R^2 = 7.44\%$).

Indices of disease activity correlated significantly with each other (table 2). There were no significant differences in either disease activity or AGP reactivity coefficient between women and men or between patients who were seropositive or seronegative.

Discussion

Reactivity of AGP with Con A in patients with different inflammatory diseases may be increased or decreased. Acute inflammation, as found in patients with acute bacterial infections or burns,^{5,9} is characterised by an increased reactivity of AGP with Con A. In contrast, decreased AGP-Con A reactivity was found in serum samples of patients with chronic inflammatory diseases, including RA.^{3,5,7,8,15}

Our results suggest, however, that the microheterogeneity of AGP in RA depends on disease duration and shows different patterns in early and established disease. Decreased reactivity of AGP with Con A is typical for longstanding RA, whereas increased binding of AGP to Con A, similar to that found in acute bacterial infections, is characteristic for early disease. This implies that RA begins as an acute inflammation and enters the chronic phase within the first year of the disease. Thus type or duration of the inflammation, or both, seem to determine the microheterogeneity of AGP. Transition from an initially increased reactivity of AGP with Con A to decreased reactivity is found when bacterial infections become chronic.¹⁶ Conversely, the early phase of basically chronic inflammatory conditions, such as RA, may be associated with increased reactivity of AGP with Con A. Our findings raise the question as to whether infectious factors play a part in the development of RA, as previously reported.¹⁷

There is evidence that cytokines have a key role in the stimulation of the production of acute phase proteins in the liver¹⁸⁻²³ and also regulate their glycosylation.^{24,25} Our findings suggest that there are differences in the composition of cytokines produced in inflamed synovium in early and longstanding RA, and that these differences are responsible for different patterns of glycosylation of AGP.

Some results suggest that reactivity coefficients correlate with disease activity in RA.⁸ We found a significant inverse correlation between AGP reactivity coefficients and either ESR ($p < 0.005$) or disease activity as measured by the Mallya-Mace activity scale ($p < 0.05$) (table 2). A rise in activity during the first months of disease might explain this finding. However, disease activity did not correlate with disease duration in the group studied.

It has also been shown that the serum

concentration of sIL-2R correlates with disease duration and disease activity in RA.²⁶⁻²⁸ Although concentrations of sIL-2R tended to be higher in patients with longer disease duration, we were unable to show a statistically significant relation between sIL-2R concentrations and disease duration. Additionally, there was no correlation between disease activity and sIL-2R concentrations. If it is assumed that sIL-2R is an indicator of lymphocyte activity, however, our findings suggest that cellular activation may be more important in the later stage of rheumatoid synovitis.

In conclusion, there is an increase in AGP-Con A reactivity in early RA and a decreased reactivity of AGP with Con A in established disease. These changes in reactivity may reflect the differences in the cytokine network at different stages of the disease, suggesting that different mechanisms may play a part in the immunopathogenesis of early and longstanding RA. Further studies are needed to establish the clinical significance of our findings.

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