Serum IgA, acute phase proteins, and glycosylation of α₁-acid glycoprotein in ankylosing spondylitis

ANDRZEJ MACKIEWICZ, MUHAMMAD A KHAN, THOMAS L REYNOLDS, SJEF VAN DER LINDEN, AND IRVING KUSHNER

From the Case Western Reserve University, Department of Medicine, Division of Rheumatology, Cleveland Metropolitan General Hospital, Cleveland, Ohio, USA and Academish Ziekenhuis Maastricht, The Netherlands

SUMMARY Several investigators have suggested that gastrointestinal inflammation has a role in the pathogenesis of ankylosing spondylitis. To test this hypothesis markers of gastrointestinal immunostimulation, as manifested by serum IgA concentrations, were compared with serum markers of inflammation, as manifested by acute phase proteins. Serum samples from 45 unrelated Caucasian patients with ankylosing spondylitis (AS) were tested for correlation of serum IgA and six acute phase proteins: C reactive protein (CRP), α₁-antitrypsin, α₁-antichymotrypsin, caeruloplasmin, α₁-acid glycoprotein (AGP), and haptoglobin. Serum IgA was shown to be significantly positively correlated with four of these six acute phase proteins: CRP (r=0.58, p<0.001), α₁-antitrypsin (r=0.29, p<0.05), AGP (r=0.61, p<0.01), and haptoglobin (r=0.58, p<0.001), suggesting that gastrointestinal immunostimulation does have a role in the pathogenesis of inflammation in AS. In addition, the microheterogeneity of the pattern of glycosylation of AGP, expressed as reactivity coefficients, was examined. The AGP reactivity coefficient has been shown to increase in infection, remain the same in systemic lupus erythematosus, and decrease in rheumatoid arthritis. It was found that the AGP reactivity coefficient was significantly decreased in patients with AS as compared with healthy controls (p<0.006). As recent studies have indicated that patterns of glycosylation reflect intrahepato-cellular biosynthetic processes induced by cytokines our data suggest that cytokine-hepatocellular mechanisms in AS may be similar to those occurring in rheumatoid arthritis, but different from those in systemic lupus erythematosus or infection.

Key words: C reactive protein, concanavalin A, cytokines.

Evidence has recently been presented suggesting that gastrointestinal inflammation, with associated gastrointestinal immune stimulation, may have a role in the pathogenesis of ankylosing spondylitis (AS) and related spondyloarthropathies. Serum concentrations of IgA may reflect such gastrointestinal immune stimulation. A correlation between serum IgA concentrations and serum markers of inflammation, such as acute phase proteins, would support the view that gastrointestinal inflammation has an important role in the pathogenesis of AS. Three recent studies have explored this subject by evaluating the degree of correlation between serum concentrations of IgA and C reactive protein (CRP), an acute phase reactant thought to correlate with clinical indices of disease activity in AS. Results have been contradictory. Although all three groups noted an increase of IgA and CRP concentrations in patients with AS, Trull et al and Collado et al reported a significant positive association whereas Sanders et al failed to show a significant correlation. Recently, interest has focused on another, qualitative, acute phase phenomenon—alteration in the pattern of glycosylation of the microheterogeneous forms of acute phase glycoproteins. This pheno-
menon may be detected as a change in the degree of binding of serum glycoproteins to concanavalin A (con A). The direction of change—that is, increase or decrease of binding to con A, may be dissociated from that of acute phase protein changes.6 7 The degree of con A binding of α1-acid glycoprotein (AGP) has been examined in inflammatory states in which variable degrees of acute phase proteins are usually seen and has been shown to increase in bacterial infection,6 to decrease in rheumatoid arthritis,7 and to remain unchanged in systemic lupus erythematosus.6 This phenomenon has not been studied in AS. Herein we report our results of such studies in AS. Firstly, to investigate the relation between gastrointestinal immunostimulation and gastrointestinal inflammation we correlated serum IgA concentrations with six acute phase proteins: CRP, AGP, α1-antichymotrypsin, α1-antitrypsin, haptoglobin, and caeruloplasmin. Secondly, we examined the patterns of change in glycosylation of the acute phase protein AGP. These findings were compared in Caucasian patients with AS and healthy controls.

**Patients and methods**

**Patients**

Serum samples from 45 patients with AS, randomly selected from a total of 303 Caucasian patients with AS, and 11 healthy individuals were studied. The patients with AS fulfilled the New York criteria8 as well as the modified criteria.9

**Methods**

Serum concentrations of IgA and CRP were determined by radial immunodiffusion.10 Serum concentrations of AGP, α1-antichymotrypsin, α1-antitrypsin, haptoglobin, and caeruloplasmin were measured by electroimmunoassay11 using human serum calibrator kit (Atlantic Antibodies, Boston, MA).

Microheterogeneity of AGP was studied using a modification of the method of Bøg-Hansen12 by two dimensional agarose affinity electrophoresis with con A as a ligand, as described in detail elsewhere.13 Briefly, 50 μM con A was included in the first dimension gel and electrophoresis was carried out (from left to right) for 60 minutes at 10 V/cm (Fig. 1). In this first dimension AGP variants with the greatest affinity for con A were bound on the left hand part of the gel; variants with less affinity travelled farther toward the right hand region. Subsequently, the gel was transferred onto the second dimension plate in order to measure the AGP variants. Two gels adjacent to the first dimension gel, one containing anti-AGP antibodies and the other (intermediate gel) containing 7.5% of α-methyl-d-mannoside, were cast. Electrophoresis in the second dimension was carried out for 16–18 hours at 1.5 V/cm. The gel was then washed, dried, and stained with Coomassie brilliant blue R250 (Sigma, St Louis, MO). This method showed three forms of AGP (Fig. 1): variant 0, the right hand precipitate curve, was non-reactive with con A; variant 1, the centre curve, was weakly reactive with con A; and variant 2, the left hand curve, was strongly reactive with con A. The area under the precipitate curves was measured by planimetry, and the relative amounts of different forms of AGP were expressed as percentages of the total. Results were expressed as an AGP-con A reactivity coefficient calculated according to the formula: (sum of con A—reactive variants)/(non-reactive variant).

**Fig. 1** Agarose affinity electrophoresis with concanavalin A (con A) of α1-acid glycoprotein (AGP) from: (a) a representative healthy individual (AGP reactivity coefficient=1.3); (b) a representative patient with ankylosing spondylitis (AGP reactivity coefficient=0.7). The precipitate curves in each of the above two representative samples are from left to right: variant 2 of AGP (strongly reactive with con A), variant 1 (weakly reactive), and variant 0 (non-reactive with con A). The location of the original sampling well can be seen at the lower left hand corner of the figure.
STATISTICS

Statistical results were calculated using 'Statworks' (Cricket Software, Philadelphia, Pennsylvania). Linear regression analysis was used to compare serum IgA concentrations with each of the six acute phase proteins in the patients with AS. To study changes in the patterns of glycosylation of AGP mean serum concentrations of AGP-con A reactivity coefficients were calculated for the healthy controls and the patients with AS. These values were subsequently compared by Student's t test.

Results

Table 1 shows that mean values of serum IgA and all six acute phase proteins studied were significantly raised in patients with AS compared with healthy controls. Figure 2 demonstrates that there was a significant positive correlation between the serum IgA and CRP concentrations in patients with AS (r=0.58, p<0.001, n=36). Serum IgA and AGP concentrations also showed a significant positive correlation (r=0.61, p<0.01, n=36) (Fig. 3). Similarly, haptoglobin and α1-antitrypsin concentrations both showed significant positive correlation with serum IgA concentrations in patients with AS (r=0.58, p<0.001, n=36 and r=0.29, p<0.05, n=36 respectively) (Figs 4 and 5). Neither caeruloplasmin (r=0.12, n=36) nor α1-antichymotrypsin (r=0.26, n=36) were significantly associated with serum IgA concentrations.

Table 1  Mean values and significance levels of serum IgA and acute phase proteins in patients with ankylosing spondylitis (AS) and in healthy controls. Values reported in mg/l as mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>Patients with AS (n=45)</th>
<th>Healthy controls (n=11)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>2756 (1244)</td>
<td>1700 (510)</td>
<td>&lt;0.008</td>
</tr>
<tr>
<td>C reactive protein</td>
<td>10.7 (15.6)</td>
<td>&lt;2 (0)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>α1-Antitrypsin</td>
<td>2723 (532)</td>
<td>2312 (454)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>α2-Antichymotrypsin</td>
<td>607 (192)</td>
<td>401 (74)</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>1551 (914)</td>
<td>750 (348)</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td>Caeruloplasmin</td>
<td>353 (69)</td>
<td>277 (58)</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>α1-Acid glycoprotein</td>
<td>909 (230)</td>
<td>666 (132)</td>
<td>&lt;0.003</td>
</tr>
</tbody>
</table>

Fig. 3  Significant positive correlation between serum IgA and α1-acid glycoprotein (AGP).

Fig. 2  Significant positive correlation between serum IgA and C reactive protein (CRP).

Fig. 4  Significant positive correlation between serum IgA and haptoglobin (Hp).
gastrointestinal inflammation may have a role in the pathogenesis of AS.

The patients with AS manifested an acute phase response, showing significant increases in serum concentrations of all six acute phase proteins tested, as would be expected in a chronic inflammatory disease. On the other hand, alteration in structure of the glycan side chains of AGP, as reflected by con A binding, could not be predicted by deduction as such changes appear to represent an independent phenomenon during the course of inflammatory states.

This patient population showed a decrease in AGP binding to con A, similar in direction, though less in magnitude, to that seen in patients with rheumatoid arthritis. 7 This contrasts with the increased AGP binding to con A observed in sera of patients with infections, whereas those with active systemic lupus erythematosus show normal con A binding, even when AGP concentrations are significantly raised. 6 Recent studies have indicated that such changes in pattern of glycosylation may reflect altered intrahepatocellular processes induced by cytokines. 14 The different patterns of change in various inflammatory diseases may thus result from production of different groups of cytokines, or differences in hepatocellular responses to cytokines in different groups of diseases—for example, acute as opposed to chronic inflammatory states.

**Fig. 5** Significant positive correlation between serum IgA and α1-antitrypsin (AT).

Mean values of AGP-con A reactivity coefficients were 1-30 (0-14) in the 11 healthy controls compared with 1-08 (0-23) in the 45 patients with AS (values reported as mean (SD)) (p<0-006).

**Discussion**

There were two major findings in this study: (a) serum IgA concentrations showed a significant positive correlation with concentrations of four acute phase proteins (CRP, AGP, haptoglobin, and α1-antitrypsin) in patients with AS; and (b) patients with AS showed decreased binding of AGP to con A compared with normal controls, reflecting altered glycan side chain structure of AGP in these patients.

It is generally held that an increase of serum IgA concentrations may reflect gastrointestinal inflammatory states with accompanying local immunostimulation. Our findings that IgA concentrations were raised in patients with AS and that these concentrations correlated with those of CRP support the view that gastrointestinal immunostimulation is occurring in patients with AS and that its magnitude is correlated with the degree of systemic inflammation in the patients. These findings are consistent with those of Trull et al 2 and Collado et al 4, but not with those of Sanders et al. 5 Of additional interest was the correlation of CRP with three other acute phase proteins: α1-antitrypsin, AGP, and haptoglobin. This suggests that the correlation seen between serum IgA and CRP is not a chance phenomenon but rather is linked to the gastrointestinal inflammatory process, as shown in the acute phase response. These data support the view that

**References**


Serum IgA, acute phase proteins, and glycosylation of alpha 1-acid glycoprotein in ankylosing spondylitis.

A Mackiewicz, M A Khan, T L Reynolds, S van der Linden and I Kushner

doi: 10.1136/ard.48.2.99

Updated information and services can be found at:

http://ard.bmj.com/content/48/2/99

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/