IgA1 and IgA2 subclass antibodies against *Klebsiella pneumoniae* in the sera of patients with peripheral and axial types of ankylosing spondylitis

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Abstract

**Objective**—To study further the Klebsiella specific serum antibody response in patients with axial and peripheral types of ankylosing spondylitis (AS).

**Methods**—IgA1 and IgA2 subclass antibodies to *Klebsiella pneumoniae* were measured by enzyme linked immunosorbent assay in the sera of 171 patients with axial or peripheral type AS, and in sera of 100 healthy controls. The effect of 26 weeks of sulphasalazine treatment on the antibody levels in the two types of AS was also analysed.

**Results**—*K pneumoniae* specific antibody levels of both IgA1 and IgA2 subclasses were increased in the sera of patients with AS compared with healthy controls. The increased levels were present in patients with axial and with peripheral AS, and there were no statistically significant differences in the antibody levels between these two groups. Sulphasalazine treatment decreased the Klebsiella specific antibody level of IgA1 subclass in patients with axial AS, but there were no statistically significant changes in the IgA2 subclass, or in the patients with peripheral type AS.

**Conclusions**—These results agree with earlier published findings suggesting that IgA (especially Klebsiella specific IgA) may have a role in the pathogenetic mechanisms of both peripheral and axial types of AS. In addition, it seems that both IgA1 and IgA2 subclasses are involved in the disease process.

The aetiology of ankylosing spondylitis (AS) remains unknown. The genetic factor HLA B27 evidently has an important role in the pathogenesis, and a role for gut and mucosal immune defence mechanisms has also been suggested—gut permeability has been shown to be increased in patients with AS, for example. In addition, serum concentrations of total IgA, and secretory IgA and the IgA subclasses, have been shown to be persistently increased in patients with AS; these observations have been interpreted as signs of stimulation of the secretory immune system, perhaps through the gastrointestinal tract. Other findings supporting the role for IgA in AS include IgA deposition in dermal vessels in biopsy specimens, and circulating IgA containing immune complexes in patients with AS. *Klebsiella pneumoniae*, a bacterium often present in normal gut flora, has been suggested to be a causative or exacerbating agent for AS. There are reports of increased faecal carriage of Klebsiella species, as well as reports of increased antibody levels, especially of IgA, against Klebsiella bacteria in patients with AS, and a clear decrease in the concentration of IgA class antibodies against *K pneumoniae* during the 26 weeks of sulphasalazine treatment has been reported.

Human IgA subclasses, IgA1 and IgA2, are characteristically distributed in serum and external secretions. Approximately 85% of serum IgA antibodies belong to the IgA1 subclass, whereas more than 50% of the IgA in external secretions belongs to the IgA2 subclass. Hence, IgA2 has been considered as a marker of mucosal origin. The serum IgA originates primarily from bone marrow, whereas the IgA in secretions is produced locally in secretory glands and tissues—mainly the gastrointestinal mucosa. The distribution of cells synthesising the different immunoglobulin subclasses is known to vary in different tissues and organs; IgA2 production is most enhanced in the distal gut. Thus determination of the IgA subclasses may provide information on the tissue origin of specific IgA antibodies.

Clinically, patients with AS can be divided into two groups: those with a pure axial form of the disease, and those with both axial and peripheral joint arthropathies. Different aetio-pathogenetic mechanisms have been proposed for these two types of AS. For example, patients with peripheral type AS seem more often to have intestinal inflammatory lesions diagnosed by ileocolonoscopy than those with the axial type of disease. In addition, sulphasalazine, a drug used to treat inflammatory bowel disease, seems to be especially effective in patients with peripheral type AS. Recently, we have shown that there are certain differences in the immunological parameters between these two AS patient groups.

It remains an open question where the increased Klebsiella specific IgA production in patients with AS takes place, and whether it differs between patients with the axial and the peripheral types of AS. We have approached the question by measuring separately the IgA1...
and IgA2 antibodies to *K. pneumoniae* by enzyme linked immunosorbent assay (ELISA) in patients with axial and peripheral types of AS. The effect of sulphasalazine treatment on the antibody levels in the two types of AS was also analysed.

**Patients and methods**

**PATIENTS**

Two previously described patient populations were included in the study: the first consisted of 98 patients with AS and the second, 73 patients with AS participating in a randomised, placebo controlled trial of sulphasalazine. Seven patients were included in both patient populations. In the first group, 65 of the patients had the pure axial form of AS (16 women and 49 men; mean age 38 years (range 18–67), and 33 also had peripheral joint arthritides (nine women and 24 men; mean age 40 years (range 24–62)). In the second patient population there were 35 patients with the peripheral (nine women and 26 men; mean age 38 years (range 24–56)) and 38 patients with the axial (nine women and 29 men; mean age 38 years (range 22–61)) type of AS. Patients selected for sulphasalazine treatment fulfilled the criteria for active disease (Westergren erythrocyte sedimentation rate (ESR) at least 30 mm/1st h or C reactive protein (CRP) at least 20 mg/l, and morning stiffness lasting longer than 30 minutes). Of the patients with peripheral AS who had paired serum samples available, 13 received sulphasalazine treatment and 18 placebo; the corresponding figures for patients with axial AS were 20 for sulphasalazine and 17 for placebo. The sulphasalazine or placebo treatment lasted for 26 weeks (2–3 g/day depending on efficacy and tolerance). There was a statistically significant improvement seen in most of the clinical variables in patients receiving sulphasalazine; at the end of the treatment, significant differences between the sulphasalazine and placebo groups were observed in morning stiffness, chest expansion, ESR, and in all immunoglobulin classes. There were five withdrawals from each group because of side effects or loss to follow up. Mild and spontaneously disappearing side effects occurred in nine patients in the sulphasalazine group and in 11 patients in the placebo group.

**SERUM SAMPLES**

Serum samples from the patients participating in the trial with sulphasalazine were collected at their first visit before sulphasalazine or placebo treatments were started, and at 26 weeks, at the end of the treatment. All sera, including control sera from 100 healthy blood donors (73 women and 27 men; mean age 38 (range 17–63)), were stored at −20°C until tested simultaneously.

**K. PNEUMONIAE ANTIGEN FOR ELISA**

Sodium dodecyl sulphate (SDS) extract of whole *K. pneumoniae* bacteria (ATCC 27736) was used as antigen in the ELISA. To prepare the antigen, 10 ml of an overnight nutrient broth culture of *K. pneumoniae* was diluted 1:40 in fresh nutrient broth and grown on a shaker for four hours at 37°C. After three washings, the bacteria were treated with 0.1% (weight/volume) SDS for one hour at 37°C to obtain an antigen extract (SDS extract).

**ELISA FOR K. PNEUMONIAE SPECIFIC ANTIBODIES OF IGA1 AND IGA2 SUBCLASSES**

*K. pneumoniae* specific antibodies of IgA subclasses IgA1 and IgA2 were measured as described earlier for Yersinia and Salmonella antibodies. Serum samples at 1:50 dilution in 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) (0.1 mol/l, pH 7.5) were allowed to react with the SDS extract antigen of *K. pneumoniae*(0.1 μg/ml) attached to polystyrene microtitre plates (Nunc, Roskilde, Denmark) overnight at room temperature. Monoclonal antibody against IgA1 or IgA2 (Beckton Dickinson, Mountain View, California) was added at a dilution of 1:100 and incubated for three hours at 37°C. Swine alkaline phosphatase conjugated antirabbit IgG (Orion Diagnostica, Espoo, Finland) at a dilution of 1:250 was added to detect bound antibodies. Fresh p-nitrophenyl phosphate in diethanolamine-magnesium chloride buffer solution (1 mg/ml) (Orion Diagnostica) was added, incubated for 30 minutes at 37°C and the reaction stopped with 1 mol/l sodium hydroxide. The optical density was measured with a Titertek Multiscan Photometer (Lab-systems, Helsinki, Finland) at a wavelength of 405 nm.

**STATISTICAL ANALYSIS**

The mean concentrations of antibodies in different groups were compared with Student’s *t* test. The effect of sulphasalazine on antibodies was analysed using the paired Student’s *t* test. The χ² test was used to evaluate associations between the diminishing ESR, CRP, severity of spinal pain or morning stiffness, and the diminishing of Klebsiella antibodies of the IgA subclasses during sulphasalazine treatment.

**Results**

In both patient populations the Klebsiella specific antibody levels of IgA1 and IgA2 subclasses were greater than those of controls (*p* < 0.0001). In addition, when the patients were grouped according to the presence or absence of peripheral arthritides (peripheral and axial types of AS), both groups in both patient populations had increased Klebsiella antibody levels of both IgA subclasses compared with controls (*p* < 0.0001). There were no statistically significant differences in the antibody levels between the patients with axial
Klebsiella pneumoniae specific antibodies of IgA1 and IgA2 subclasses in the sera of patients with peripheral and axial types of ankylosing spondylitis (AS) and of healthy controls

<table>
<thead>
<tr>
<th>Population</th>
<th>IgA1</th>
<th>IgA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mäki-Ikola et al.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients (n = 98)</td>
<td>0.312 (0.158)</td>
<td>0.184 (0.101)</td>
</tr>
<tr>
<td>Peripheral AS (n = 33)</td>
<td>0.332 (0.149)</td>
<td>0.203 (0.112)</td>
</tr>
<tr>
<td>Axial AS (n = 65)</td>
<td>0.302 (0.163)</td>
<td>0.175 (0.094)</td>
</tr>
<tr>
<td>Nissilä et al.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients (n = 73)</td>
<td>0.560 (0.159)</td>
<td>0.297 (0.109)</td>
</tr>
<tr>
<td>Peripheral AS (n = 35)</td>
<td>0.544 (0.157)</td>
<td>0.317 (0.128)</td>
</tr>
<tr>
<td>Axial AS (n = 38)</td>
<td>0.574 (0.161)</td>
<td>0.279 (0.085)</td>
</tr>
<tr>
<td>Controls (n = 100)</td>
<td>0.171 (0.117)</td>
<td>0.074 (0.071)</td>
</tr>
</tbody>
</table>

Values are mean (SD) of absorbance values at optical density 405 nm.
All values for both patient groups in both patient populations in IgA1 and IgA2 subclasses are greater than those for controls (p < 0.0001); no statistically significant differences between peripheral and axial patient groups.
All values for Nissilä’s patients in IgA1 and IgA2 subclasses are greater than those for Mäki-Ikola’s patients (p < 0.0001, except p = 0.0002 for peripheral AS in IgA2).

or peripheral types of AS in either of the patient populations. Comparing the first patient population (of Mäki-Ikola et al.12) with the second patient population (of Nissilä et al.25), the latter population had always statistically significantly greater antibody levels in both IgA subclasses (table).

The effect of sulphasalazine or placebo on the Klebsiella specific antibody levels of IgA subclasses was studied in the patients of Nissilä et al.25 (figs 1, 2). There were no changes in IgA1 or IgA2 antibody levels between the baseline and 26 week samples in patients receiving placebo. However, in patients receiving sulphasalazine the IgA1 antibody level decreased significantly during the 26 week follow up (in 33 paired samples, from OD405 of 0.571 (SD 0.151) at baseline to OD405 0.528 (0.180) at 26 weeks; p = 0 < 0.04), whereas no change was seen in the IgA2 antibody level.

Further, when the patients were grouped according to the presence or absence of peripheral arthritis, the IgA1 antibody level decreased significantly only in the axial type of AS (in 20 paired samples, from OD405 0.564 (0.172) at baseline to OD405 0.490 (0.177) at 26 weeks; p < 0.02), whereas no statistically significant changes in levels of the IgA2 subclass were seen.

No statistically significant correlations were seen in patients with axial or with peripheral AS, between the decrease in IgA1 and IgA2 subclass Klebsiella antibody levels and the severity of spinal pain, the severity of morning stiffness, ESR, or CRP (these variables were earlier shown to characterise best the activity of AS25). However, in patients with axial AS that were included into the present study, the severity of spinal pain (assessed by visual analogue scale), the severity of morning stiffness (assessed by visual analogue scale), ESR, and CRP seemed to decrease more during the sulphasalazine treatment than they did in patients with peripheral AS (mean changes, respectively: −15, −22, −14 and −20 in axial group, and −11, −8, −7 and −14 in peripheral group).

Discussion

In the present study we have shown that K pneumoniae specific antibody levels of both IgA1 and IgA2 subclasses were increased in the sera of patients with AS compared with healthy controls. In addition, the increased IgA1 and IgA2 Klebsiella specific antibody levels were seen both in patients with axial and in patients with peripheral type AS, but there were no statistically significant differences in the antibody levels between these two types of AS. This corroborates findings of our recent study in which an increased IgA class Klebsiella specific antibody level was found in both types of AS in these same patients.16 Thus these findings support earlier published results emphasising the importance of IgA class antibodies, and especially of those with specificity to K pneumoniae, in the pathogenesis of AS.1

The presence of increased Klebsiella specific IgA subclass antibody levels in the sera of patients with AS may be related to infection, inflammation, or change in the mucosal permeability in the gut. The increased IgA1 Klebsiella specific antibody level in patients...
with AS suggests a non-secretory origin for the increased total serum IgA, which must be ascribed to the central immune system.\textsuperscript{17} 18 20-22 However, the Klebsiella antibody level of IgA2 subclass was also increased, suggesting a contribution by the mucosa (for example gut) to the increased IgA production.\textsuperscript{17} 18 20-22 The latter observation is of special interest, as the relation of the gut to inflammatory joint manifestations has received much attention recently. In patients with AS, both ileal and colonic mucosa often show macroscopic and microscopic signs of inflammation, which may resemble an early form of Crohn’s disease.\textsuperscript{23} 27 In addition, gastrointestinal infections caused by Yersinia and Salmonella may be complicated by reactive arthritis, when causative microbial antigens (lipopolysaccharide) are transported to the joints.\textsuperscript{28} 30 Furthermore, in reactive arthritis the causative microbe may persist in the body, for example in the gut, and thus result in increased and persisting antibody production, especially of the IgA class.\textsuperscript{18} 28 29 Thus, if the pathogenetic mechanisms for both AS and reactive arthritis are the same, Klebsiella infection in the gut may be a causative factor for the previously described enhanced permeability of the gut in patients with AS,\textsuperscript{3} similar to that seen after Yersinia enteritis.\textsuperscript{31} This could then allow certain antigenic material, such as Klebsiella lipopolysaccharide, to pass through the mucosa into the circulation, to be transported thereafter into the sites of inflammation in AS—axial, or peripheral joints, or both. Consequently, the IgA1 and IgA2 class antibodies, at least, are produced as a result of mucosal and systemic antigenic stimulation. However, in a recent study O’Mahony et al failed to find any evidence for abnormal intestinal IgA antibody response to Klebsiella in patients with AS.\textsuperscript{32}

The patient population reported by Nissilä et al\textsuperscript{25} had greater levels of the IgA subclass Klebsiella specific antibody compared with the patient population reported by Mäki-Ikola et al.\textsuperscript{12} This may reflect the fact that all of the patients in the study by Nissilä’s group had clinically active disease, whereas the clinical condition of patients in the other study varied from inactive to active.

Sulphasalazine treatment decreased the Klebsiella specific antibody level of the IgA1 subclass in the patients with axial AS, but no statistically significant changes were seen in the IgA2 subclass or in patients with peripheral AS. Earlier, we have shown that the total IgA class antibody level against the whole Klebsiella bacteria declined statistically significantly during the sulphasalazine treatment,\textsuperscript{19} but when the patients were divided into the two groups (axial and peripheral types of AS), the statistical significance was lost.\textsuperscript{19} However, when Klebsiella lipopolysaccharide specific antibodies were analysed, the decline in IgA antibody level during the sulphasalazine treatment was more pronounced and also statistically significant in both types of AS,\textsuperscript{16} indicating an important role for lipopolysaccharide in the pathogenesis. The fact that the Klebsiella IgA subclass antibody levels did not correlate directly with the changes in clinical severity of the disease argues against a direct role for Klebsiella in determining flare of pre-existing disease. However, it is interesting that when the present patients with axial type AS were analysed as a group, the IgA1 antibody level decreased statistically significantly only in this group treated with sulphasalazine, and with the most significant improvement in clinical condition. Therefore, the present observations of the decline of Klebsiella antibodies of IgA1 subclass in patients with axial type AS, especially, are of interest and warrant further studies.

It cannot be excluded that use of non-steroidal anti-inflammatory drugs (NSAID) in the present patients may have had an influence on antibody levels because of their tendency to increase gut permeability,\textsuperscript{33} as the majority of our patients were taking NSAIDs. Further, during amelioration of the symptoms of AS, the patients reduced their use of NSAIDs, which could have led to a change in mucosal permeability towards normal. This might also have contributed to the decrease of IgA2 subclass antibodies from the gut and led to a decrease in the antibody levels. However, no significant differences were recently seen in the frequency of endoscopically visualised lesions between patients with and without prior NSAID use.\textsuperscript{27} Furthermore, this does not explain the fact that only in the patients with the axial type of AS did the Klebsiella IgA1 subclass antibody levels decrease statistically significantly, as there were no differences in the use of NSAIDs between the two patient groups.

In conclusion, our present results are in agreement with the earlier findings which suggested that IgA (especially Klebsiella specific IgA) may play a role in the pathogenetic mechanisms of both peripheral and axial types of AS. In addition, it seems that both IgA1 and IgA2 subclasses are involved in the disease process.

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