IgA CLASS SERUM ANTIBODIES AGAINST THREE DIFFERENT KLEBSIELLA SEROTYPES IN ANKYLOSING SPONDYLITIS

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SUMMARY

Objective. To investigate the possible predominance of certain Klebsiella pneumoniae capsular types in the pathogenesis of ankylosing spondylitis (AS).

Methods. The prevalence of IgA class antibodies against three different K. pneumoniae strains (with capsular types 21, 30 and 43) was studied in the sera of 177 patients with AS and of 100 healthy blood donors using an enzyme-linked immunosorbent assay.

Results. The median Klebsiella-specific antibody levels were always higher in patients than in controls regardless of the serotype used as antigen. When the prevalence of increased antibody levels was compared between the groups, it was highest against the strain with capsular type 30, whereas against strains 21 and 43 it was similar among patients and controls.

Conclusions. A broad range of Klebsiella serotypes may be involved in the pathogenesis of AS. Thus, it is important to take the different Klebsiella serotypes into particular account in these studies.

Key words: Ankylosing spondylitis, Antibody, Klebsiella pneumoniae, ELISA, Capsular type.

The aetiology of ankylosing spondylitis (AS) is still unknown. The genetic factor HLA-B27 evidently has an important role in its pathogenesis. Furthermore, Klebsiella pneumoniae, a bacterium often present in normal gut flora, has been suggested to be a causative or exacerbating agent for AS [1]. 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 [1–11]. Recently, strong direct evidence for an abnormal mucosal humoral immune response, particularly to K. pneumoniae, in patients with AS was also found by studying jejunal fluids from AS patients obtained by a double-balloon perfusion device [12]. Further, a quantitative reduction of K. pneumoniae-responsive T cells in peripheral blood of AS patients may reflect a defective peripheral T-cell defence in the immune response to Klebsiella [13].

Serologically, Klebsiella species are classified into 77 different capsular types [14]. Although it has been shown that some Klebsiella capsular serotypes are isolated at a significantly higher frequency than others in different infections, there is no apparent correlation between the capsular type and any particular isolation site or disease state [15, 16]. Previously, only one group has studied the possible predominance of certain Klebsiella capsular types in AS patients [6, 7]. The present study was undertaken to investigate whether the prevalence of Klebsiella-specific antibodies in Finnish AS patients varies when different Klebsiella serotypes are used as antigens in an enzyme-linked immunosorbertent assay (ELISA).

METHODS

Patients and controls

Two earlier described patient populations were included in the study [8, 10, 17]. The first patient population consisted of 99 patients with AS (74 males and 25 females with a mean age of 39 yr) [8] and the second patient population of 85 patients with AS participating in a randomized, placebo-controlled trial of sulphasalazine (67 males and 18 females with a mean age of 38 yr) [10, 17]. Clinical characteristics of the patients have been described in detail earlier [8, 17]. There were seven patients who had been included in both patient materials. All sera, including control sera from 100 healthy blood donors (partly different populations in different assays), were stored at −20°C until tested simultaneously. For technical reasons, the prevalence of increased antibody levels in the two AS patient groups was analysed separately (also using partly different control samples) in the assay for Klebsiella 27736.

ELISA for K. pneumoniae-specific antibodies

Sodium dodecyl sulphate (SDS) extracts of K. pneumoniae strain 21, 43 and ATCC 27736 (with the capsular antigens 21, 43 and 30, respectively) were used. These strains were selected to be used as antigens for the following reasons: antibodies raised against strains 21 and 43 have been shown to lyse the HLA-B27-positive lymphocytes of ~80% of patients with AS, 60% of those with Reiter’s syndrome or B27-positive asymmetrical peripheral arthritis and 20% of patients with B27-positive uveitis; the B27-positive lymphocytes of clinically normal individuals were not lysed [18]. Strain ATCC 27736 has been widely used in our earlier studies concerning the pathogenesis of AS [8–10, 12]. The antigen extracts were prepared as previously described [8].

IgA class antibodies against the three different

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K. pneumoniae strains were analysed as described earlier [8, 10]. The polystyrene microtitre plates (Nunc, Roskilde, Denmark) were coated with SDS extracts of K. pneumoniae in phosphate-buffered saline (PBS; 0.1 mol/l, pH 7.5; 100 µl/well) overnight at 37 °C. The plates were saturated with 1% bovine serum albumin in PBS (BSA-PBS; 100 µl/well). Patient serum samples at 1:250 dilution (75 µl/well) were incubated on the plates for 2 h at 37 °C. Thereafter, 75 µl/well of alkaline phosphatase-conjugated swine anti-human IgA (Orion Diagnostica, Espoo, Finland), diluted 1:250, were incubated on the plates overnight at room temperature. Fresh p-nitrophenyl phosphate in diethanolamine–MgCl2–buffer solution (1 mg/ml; Orion Diagnostica) was added, incubated for 30 min at 37 °C and the reaction stopped with 1 m sodium hydroxide. The optical density was measured with a Titertek Multiscan Photometer (Labsystems, Helsinki, Finland) at a wavelength of 405 nm. Antibody concentrations were expressed as enzyme immunoassay units (EIU): 1 EIU was 1/100 of the corresponding antibody concentration in the positive reference serum. The median Klebsiella-specific antibody levels were compared between patients and controls by the Mann–Whitney test. Also, the prevalence of increased antibody levels was compared using three different cut-off values: values exceeding the mean EIU for the blood donors by 2, 4 or 6 s.d. The prevalence of increased Klebsiella-specific serum antibodies was compared between patients and controls by the χ² test. This additional analytical approach was chosen to allow comparison with the prevalence of increased antibody levels against other microbes as published earlier by us [8].

RESULTS

When the median Klebsiella-specific antibody levels were compared between patients and controls, the levels were always higher in patients regardless of the serotype used as antigen (Table I). The difference was smallest against K. pneumoniae 21 strain.

When an antibody concentration exceeding the mean for blood donors by 2 s.d. was defined as a positive antibody level, increased antibody levels against K. pneumoniae 27736 were observed in 12 out of 99 (12%) AS patients and 16 out of 85 (19%) AS patients [8–10]. However, when the jejunal fluid antibody levels K. pneumoniae 27736 were observed in 12 out of 99 antibody level, increased antibody levels against the whole bacterium, as well as a decrease in s.d. K. pneumoniae serotype used as antigen (Table I). The different cut-offs were compared between patients and controls, the arthritis (RA) [19]. This could have suggested intra-articular antibody production against this particular serotype in AS patients [19]. Further, in our earlier studies on Klebsiella antibodies in AS, we have mainly used the Klebsiella 27736 strain and found increased IgG, IgA and IgA subclass antibody levels against the whole bacterium, as well as a decrease in these antibody levels during sulphasalazine treatment [8–10]. However, when the jejunal fluid antibody levels in AS patients were studied against the three K. pneumoniae serotypes 21, 30 and 43, the levels were relatively similarly increased against all three serotypes [12]. Sahly et al. [6, 7] measured antibodies against

DISCUSSION

There is strong evidence that mucosal immune defence mechanisms, possibly against K. pneumoniae specifically, play an important role in the pathogenesis of AS [1–13]. However, studies considering the possible differences in the role of the different Klebsiella capsular types in AS have been reported earlier by only one group [6, 7]. In the present study, we confirmed the findings by Sahly et al. [6, 7] that there seem to be some differences in the prevalence of antibodies against different Klebsiella strains in AS patients. In the present Finnish AS patients, the median antibody levels against klebsiellae were always higher in patients than in healthy blood donors, regardless of the serotype concerned. When the prevalence of increased antibody levels was compared between patients and controls, it seemed to be highest against the ATCC strain 27736 with capsular type 30, whereas the prevalences of increased antibody levels against strains 21 and 43 were similar among patients and controls.

It is interesting that the increased antibody levels in these Finnish patients were found especially against Klebsiella strain 27736, as we have recently shown that the values reflecting intra-articular IgA and IgG class antibody production were significantly higher in AS patients only against this strain, but not against strains 21 and 43, when compared to patients with rheumatoid arthritis (RA) [19]. This could have suggested intra-articular antibody production against this particular Klebsiella type in AS patients [19]. Further, in our earlier studies on Klebsiella antibodies in AS, we have mainly used the Klebsiella 27736 strain and found increased IgG, IgA and IgA subclass antibody levels against the whole bacterium, as well as a decrease in these antibody levels during sulphasalazine treatment [8–10]. However, when the jejunal fluid antibody levels in AS patients were studied against the three K. pneumoniae serotypes 21, 30 and 43, the levels were relatively similarly increased against all three serotypes [12]. Sahly et al. [6, 7] measured antibodies against

**TABLE I**

<table>
<thead>
<tr>
<th>K. pneumoniae 27763</th>
<th>K. pneumoniae 21</th>
<th>K. pneumoniae 43</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.7 (15.7–22.7)</td>
<td>28.5 (20.4–45.9)</td>
<td>8.8 (5.5–14.8)</td>
</tr>
<tr>
<td>Controls ($n = 100$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.9 (11.5–22.6)</td>
<td>14.6 (9.1–22.7)</td>
<td>8.32 (5.4–12.5)</td>
</tr>
<tr>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.03$</td>
</tr>
</tbody>
</table>

The median (interquartile range; 25th and 75th percentiles) is given.
TABLE II
Prevalence of increased IgA class antibody levels against three Klebsiella pneumoniae strains in the sera of patients with ankylosing spondylitis and of controls (healthy blood donors)

<table>
<thead>
<tr>
<th></th>
<th>K. pneumoniae 27736</th>
<th>K. pneumoniae 21</th>
<th>K. pneumoniae 43</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AS patients (n = 99)</td>
<td>Controls (n = 100)</td>
<td>AS patients (n = 177)</td>
</tr>
<tr>
<td>With antibody concentrations exceeding the mean for blood donors by 2 s.d.</td>
<td>12 (12)*</td>
<td>4 (4)</td>
<td>16 (19)</td>
</tr>
<tr>
<td></td>
<td>P = 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With antibody concentrations exceeding the mean for blood donors by 4 s.d.</td>
<td>1 (1)*</td>
<td>8 (9)</td>
<td>3 (3)</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With antibody concentrations exceeding the mean for blood donors by 6 s.d.</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>7 (8)</td>
</tr>
</tbody>
</table>

*Published earlier by Mäki-Ikola et al. [8].

References

Acknowledgements
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