Bacterial antibodies in ankylosing spondylitis

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(Accepted for publication 17 December 1990)

SUMMARY
Antibodies to Salmonellae, Yersiniaiae, Campylobacter jejuni, Borrelia burgdorferi, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis and Chlamydia trachomatis were measured by ELISA in the sera of 99 patients with ankylosing spondylitis. Increased prevalence of IgA and IgG class antibodies against K. pneumoniae and of IgA class against E. coli was observed in ankylosing spondylitis. No clear correlation between the disease activity and occurrence of antibodies was revealed. The results are in line with the previously published findings suggesting that K. pneumoniae may have a role in the aetiopathogenesis of ankylosing spondylitis.

Keywords ankylosing spondylitis bacterial antibodies Klebsiella ELISA

INTRODUCTION
Certain infections, like those caused by Yersiniaiae, Salmonellae, Campylobacter jejuni, Chlamydia trachomatis and Borrelia burgdorferi, are sometimes followed by development of reactive arthritis, especially in patients carrying HLA-B27 (Toivanen & Toivanen, 1988; Weyand & Goronzy, 1989). HLA-B27 is also present in more than 90% of patients with ankylosing spondylitis (Brewerton et al., 1973). It has been proposed that this common genetic predisposition reflects common aetiopathogenetic mechanisms for both diseases. Thus, the pathological processes associated with ankylosing spondylitis may be triggered in a similar immunological manner as in the patients with reactive arthritis; in ankylosing spondylitis, Klebsiella pneumoniae has been suspected as a causative agent (Ebring et al., 1978; Ebringer, Baines & Ptaszynska, 1985a; Kinsella, 1985). To explore this possibility further, including the potential role of other microbes, we have measured IgM, IgA and IgG class antibodies to Salmonellae, Yersiniaiae, C. jejuni, B. burgdorferi, K. pneumoniae, Escherichia coli, Proteus mirabilis and Chlamydia trachomatis by ELISA in patients with AS, and correlated the positive antibody levels with disease activity.

PATIENTS AND METHODS
Ninety-nine patients treated for ankylosing spondylitis at the Rheumatism Foundation Hospital, Heinola, were included. The criteria for ankylosing spondylitis were the Rome criteria (Kellgren, Jeffrey & Ball, 1963). The patients were divided into two groups according to disease activity as assessed by the sum of values for erythrocyte sedimentation rate (ESR; normal range 1–10 mm/h) and C-reactive protein (CRP; normal range < 10 mg/l) (Kaarela, 1985). The groups were: (i) patients with inactive disease (ESR + CRP < 30; 37 patients); and (ii) patients with active disease (ESR + CRP > 30; 62 patients). Ages of the patients ranged from 18 to 67 years (mean 39), and the male-to-female ratio was 74:25. Peripheral blood lymphocytes of 29 patients were typed for HLA B27 antigen by a two-stage microlymphocytotoxicity test (Histognost-B27, Behring Institut, Behringwerke, Marburg, Germany); 28 patients were HLA B27 positive.

All sera, including control sera from 100 healthy blood donors, were stored at −20°C until tested simultaneously. Antibodies to Salmonella enteritidis and S. typhimurium were measured by ELISA using combined lipopolysaccharides as antigens (Isomäki, Vuento & Granfors, 1989); to Yersinia enterocolitica 0:3, Y. enterocolitica 0:9, Y. pseudotuberculosis I, Y. pseudotuberculosis III, K. pneumoniae (ATCC 27736), E. coli and P. mirabilis using SDS extracts of corresponding bacteria as antigens (Granfors et al., 1980, 1989b; Mäki-Ikola et al., 1991); to C. jejuni using acid glycine extract antigen (Kosunen et al., 1983); to B. burgdorferi using sonic extract antigen (Viljanen & Punnonen, 1989) and to Ch. trachomatis using elementary bodies as antigen (Finn, Ohlin & Schachter, 1983; IgM class Chlamydia antibodies were not measured because of inapplicability of the test). Antibody concentrations were expressed as enzyme immunoassay units (EIU): 1 EIU as 1/100 of the corresponding antibody concentration in the reference serum. EIU of sera from 100 healthy blood donors, i.e. same subjects as controls, were measured separately and values at least 2 s.d. higher than the mean EIU of the 100 blood donor sera were defined as positive. In Chlamydia IgA ELISA the sera were twofold diluted, starting from the dilution of 1/20, and the dilution
Table 1. Bacterial antibodies in the sera of patients with ankylosing spondylitis and of controls (healthy blood donors)

<table>
<thead>
<tr>
<th>Group</th>
<th>Salmonellae</th>
<th>Yersinia*</th>
<th>Campylobacter</th>
<th>Borrelia</th>
<th>Klebsiella</th>
<th>E. coli</th>
<th>Proteus</th>
<th>Chlamydia</th>
<th>Total no. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM IgA IgG Any IgM IgA IgG Any IgM IgA IgG Any IgM IgA IgG Any IgM IgA IgG Any IgM IgA IgG Any IgM IgA IgG Any IgM IgA IgG Any</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>2 4 3 7 19 8 14 31 3 6 1 8 1 8 1 10 7 12 22 35 4 14 4 4 10 8 7 41</td>
<td>14 9 17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>6 7 5 14 13 12 17 31 5 5 3 11 4 5 4 12 9 4 8 19 4 6 6 13 5 7 5 16</td>
<td>15 12 19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

With antibody concentrations exceeding the mean for 100 blood donors by 2 s.d.†

- Patients
- Controls

With antibody concentrations exceeding the mean for 100 blood donors by 4 s.d.

- Patients
- Controls

* Analysed separately for Y. enterocolitica O:3 and O:9, Y. pseudotuberculosis I and III.
† For Chlamydia antibodies, the following limits were applied: OD (at 492 nm) >0.3 in 1/160 dilution for IgA, and >15 EU for IgG.
‡ P < 0.05 versus controls.
§ P < 0.01 versus controls.
giving OD (at 492 nm) > 0.3 was considered as a positive titre. In Chlamydia IgG ELISA the antibody concentration > 15 EIU was considered positive.

The occurrence of antibodies in different groups was compared with the χ²-test.

RESULTS

Increased antibody levels in ankylosing spondylitis patients were most often observed against K. pneumoniae (in 35 out of 99 patients) and Yersiniae (31 patients), followed in frequency by antibodies against P. mirabilis (21 patients), E. coli (18 patients), Ch. trachomatis (17 patients), B. burgdorferi (10 patients), C. jejuni (eight patients) and Salmonellae (seven patients). The corresponding numbers for controls were 19 out of 100 for Klebsiella (P < 0.05), 31 for Yersinia, 16 for Proteus, 13 for E. coli, 19 for Chlamydia, 12 for Borrelia, 11 for Campylobacter and 1 for Salmonellae. These figures apply for antibody concentrations exceeding the mean value of blood donors by 2 s.d. (Table 1). Of the 71 patients with increased levels of bacterial antibodies, 33 (46%) had antibodies against only one, and 20 (28%) against three or more of the microbes tested. Similarly, of the 35 patients with antibodies against Klebsiella, nine (26%) had only Klebsiella antibodies, whereas 14 (40%) had antibodies against three or more of the microbes. No clear evidence was obtained to indicate that the antibodies observed would be due to polyclonal stimulation.

When taken by immunoglobin class, IgA (P < 0.05) and IgG (P < 0.01) antibodies against Klebsiella were observed significantly more often in the patients than in the healthy controls; this applies for antibody concentrations exceeding the mean values of blood donors by 2 s.d. (Table 1). In addition, when the antibody concentrations exceeding the mean values of blood donors by 4 s.d. are considered, the patients had increased prevalence of IgA class antibodies against E. coli (P < 0.05). The results for other microbes tested did not differ between the patients and the controls.

When the results are analysed according to the disease activity, no clear picture emerges (Table 2). Ten out of 62 patients with active disease had IgA class antibodies to Klebsiella (P < 0.05, when compared with four out of 100 in the healthy controls), whereas 12 out of 37 patients with inactive ankylosing spondylitis had IgG class Klebsiella antibodies, compared with 10 out of 62 with active disease or to eight out of the 10 healthy controls (P < 0.001). Six of the 37 patients with inactive disease had E. coli antibody concentrations exceeding the mean of blood donors by 4 s.d., compared with only one out of 100 of the controls (P < 0.005). Regarding the other microbes tested, no associations between the disease activity and occurrence of antibodies were observed.

DISCUSSION

In the present study prevalence of IgA and IgG class Klebsiella antibodies was elevated in ankylosing spondylitis patients, as reported previously (Trull et al., 1983, 1984; Trull & Panayi 1983; Ebringer et al., 1985a, 1985b; Cooper et al., 1988). In addition, IgA class antibodies against E. coli were also observed slightly elevated in ankylosing spondylitis; this can perhaps be explained by cross-reactive antigens with Klebsiella. Immune response to Campylobacter, Chlamydia and Borrelia in patients with ankylosing spondylitis did not differ from healthy controls. Furthermore, no differences between these two groups emerged when occurrence of Salmonella, Yersinia and Proteus antibodies were measured, as has also been reported earlier (Trull et al., 1984; Ebringer et al., 1985b; Toivanen et al., 1986; van Bohemen et al., 1986). No such a clear correlation between the disease activity and the occurrence of Klebsiella antibodies was revealed in our study as in those by other investigators (Trull & Panayi, 1983; Trull et al., 1983, 1984; Ebringer et al., 1985b).

In Yersinia-triggered reactive arthritis, Yersinia-specific IgA class antibodies persist for long period, even for several years (Granfors et al., 1980; Toivanen et al., 1987), probably reflecting

<table>
<thead>
<tr>
<th>Table 2. Klebsiella- and E. coli-specific antibodies in the sera of patients with ankylosing spondylitis, according to disease activity, and of the controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of subjects with antibodies against</strong></td>
</tr>
<tr>
<td><strong>Klebsiella</strong></td>
</tr>
<tr>
<td>Group</td>
</tr>
<tr>
<td>With antibody concentrations exceeding the mean for 100 blood donors by 2 s.d.</td>
</tr>
<tr>
<td>Active disease*</td>
</tr>
<tr>
<td>Inactive disease†</td>
</tr>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>With antibody concentrations exceeding the mean for 100 blood donors by 4 s.d.</td>
</tr>
<tr>
<td>Active disease*</td>
</tr>
<tr>
<td>Inactive disease†</td>
</tr>
<tr>
<td>Controls</td>
</tr>
</tbody>
</table>

* Sum of ESR and CRP > 30.
† Sum of ESR and CRP ≤ 30.
‡ P < 0.05 versus controls.
§ P < 0.001 versus controls.
¶ P < 0.005 versus controls.
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