IgG and IgA immune response against klebsiella in HLA-B27-associated anterior uveitis

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SUMMARY  Enteric infections with Gram-negative bacteria are thought to play an important part in HLA-B27-associated disease such as Reiter's syndrome and reactive arthritis. But the role of bacterial infections in HLA-B27-positive ankylosing spondylitis (AS) and acute anterior uveitis (AU) is still controversial. A special interest has recently been devoted to the role of klebsiella infection in HLA-B27-associated disease. We studied the humoral immune response against a 'cross-reactive' strain of Klebsiella pneumoniae in 62 patients with anterior uveitis and 33 healthy controls. The anterior uveitis patients were subdivided into 25 HLA-B27-negative patients without AS (B27+ AU+ AS-), 17 HLA-B27-positive patients without ankylosing spondylitis (B27+ AU+ AS-), and 19 HLA-B27-positive patients with ankylosing spondylitis (B27+ AU+ AS+). Total serum IgA was higher in patients than in controls in both the B27+ AU+ AS+ and B27+ AU+ AS- patients but not in the B27+ AU+ AS- group. No abnormalities were observed in the total serum IgG levels. The level of both the IgG and IgA klebsiella antibodies did not differ in the various patient groups tested as compared with the controls. Comparisons between the patient groups showed that the IgG anti-klebsiella response was higher in B27-positive patients without AS than in those with AS. These results suggest that stimulation of mucosal surfaces may play a role in HLA-B27-associated anterior uveitis. Whether klebsiella organisms are involved in this stimulation remains unclear.
patients without ankylosing spondylitis than in patients with ankylosing spondylitis.

**Materials and methods**

A total of 62 patients with anterior uveitis from the Ophthalmology Departments of the Erasmus University of Rotterdam, University of Amsterdam, and Free University of Amsterdam were included in this study. Serum was obtained from all patients during while the disease was active and from 33 healthy members of our institute.

All patients were seen by a rheumatologist. Physical examination included measuring the flexion capability of the lumbar spine by the modified technique of Schober (normal shift >5 cm) and examination of spinal movement in three planes. Chest expansion was determined at nipple level in males and below the breasts in females (normal shift >5 cm). X-rays of the sacroiliac joints were taken in anteroposterior view of the pelvis in a 25° cranio-caudal direction. All radiographs were examined without the investigator's knowing which patient's radiograph he was observing. The diagnosis of ankylosing spondylitis was made according to the New York criteria. A**

All patients were typed for the HLA-B27 antigen according to the National Institute of Health technique with four different commercially available HLA-B27 typing sera (Behring, Marburg, W Germany, one serum; Biotest, Frankfurt, W Germany, three sera). Patients and controls were subsequently divided in four groups consisting of healthy controls, B27-positive anterior uveitis patients with ankylosing spondylitis, B27-positive anterior uveitis patients without ankylosing spondylitis, and HLA-B27-negative anterior uveitis patients.

Total IgA and IgG serum levels were determined by radial immunodiffusion with precipitating rabbit antisera and a human standard serum containing 125 international units of IgG and 116 international units of IgA obtained from the Central Laboratory of Blood Transfusion (Amsterdam). IgA and IgG antibodies against klebsiella antigens were determined by an ELISA as described by Trull et al.** with minor modifications.

The klebsiella K43 'cross-reactive' strain (obtained from Dr A F Gecey, Sydney, Australia) was grown in a Kosser's citrate broth with 1% inositol** overnight at 37°C. The protein content of the culture filtrate, obtained after centrifugation and passage through Millipore filters was measured by the Folin method using bovine serum albumin (BSA) as a standard. The filtrate was diluted to a protein concentration of 7-5μg/ml in phosphate buffered saline, and micro-

**Results**

The sex and age distributions of the various patient groups and controls are shown in Table 1. Serum IgA was significantly increased in the HLA-B27-positive anterior uveitis patients also suffering from ankylosing spondylitis as compared with controls (p<0.001). The IgA levels in patients with HLA-B27-positive anterior uveitis without ankylosing spondylitis was somewhat less but still significantly (p<0.025) increased as compared with controls. Other comparisons between the groups investigated did not show significant differences.

The IgA response against klebsiella was not statistically different between the groups of patients tested and controls. When the relative amount of IgA anti-klebsiella antibodies was calculated (ratio of anti-klebsiella IgA divided by total IgA) for each patient, it was found that the HLA-B27-positive anterior uveitis patients with ankylosing spondylitis had a significantly (p<0.01) decreased ratio (Table 1). The ratios in the other patient groups were not different from those of the controls.

Serum levels of IgG (Table 2) were not altered in the patient groups investigated when compared with controls. Further, no differences were observed in the IgG anti-klebsiella antibodies between the
Table 1  Total serum IgA and IgA and klebsiella in anterior uveitis*

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Female</th>
<th>Male</th>
<th>Mean age</th>
<th>Total IgA</th>
<th>Anti-klebsiella IgA</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>33</td>
<td>19</td>
<td>14</td>
<td>34</td>
<td>109±59</td>
<td>9.5±7.5</td>
<td>0.103±0.086</td>
</tr>
<tr>
<td>B27* AU+ AS+</td>
<td>19</td>
<td>8</td>
<td>11</td>
<td>33</td>
<td>181±76</td>
<td>7.2±3.7</td>
<td>0.050±0.037</td>
</tr>
<tr>
<td>B27* AU+ AS*</td>
<td>17</td>
<td>7</td>
<td>10</td>
<td>33</td>
<td>154±84</td>
<td>9.0±6.0</td>
<td>0.073±0.069</td>
</tr>
<tr>
<td>B27* AU+ AS*</td>
<td>25</td>
<td>16</td>
<td>9</td>
<td>42</td>
<td>119±51</td>
<td>8.9±5.8</td>
<td>0.086±0.055</td>
</tr>
</tbody>
</table>

*pTotal IgA levels are expressed as international units, whereas anti-klebsiella IgA is expressed in local ELISA units. Data represent the mean ± standard deviation. Ratios represent anti-klebsiella IgA divided by total IgA as calculated for each individual.

†p<0.001 (compared with controls).
‡p<0.025 (compared with controls).
§p<0.01 (compared with controls).

patient groups and controls. The IgG anti-klebsiella response was, however, significantly higher in the B27* AU+ AS+ group as compared with the B27* AU+ AS* group (p<0.01). Calculation of the ratio between klebsiella IgG antibodies and total serum IgG showed no differences between the patient groups and controls (Table 2). A comparison of these IgG ratios between the patient groups showed that the B27* AU+ AS+ patient group had relatively less IgG anti-klebsiella antibodies compared with the B27* AU+ AS* group.

Discussion

This study shows that the total serum IgA level is increased in patients with HLA-B27-positive anterior uveitis. Similar findings were recently reported by McCoy et al.10 These observations indicate that antigenic mucosal stimulation may play a part in the pathogenesis of HLA-B27-associated anterior uveitis. Within this patient group those patients who also had ankylosing spondylitis had higher IgA levels than those without. Raised total IgA levels in ankylosing spondylitis have been reported elsewhere and were shown to be correlated with disease activity.14 In ankylosing spondylitis it has also been shown that IgA antibodies against klebsiella were increased in patients with active disease.15 Using a similar technique, we were unable to find an alteration in the IgA response against a 'cross-reactive' Klebsiella pneumoniae strain in HLA-B27-positive uveitis patients. These findings indicate that the alleged stimulatory effect on the IgA synthesis seen in B27-positive uveitis patients does not result in a parallel increase in the IgA anti-klebsiella antibodies.

Several explanations are possible for these observations. First, it can be supposed that klebsiella organisms are not involved in the course of the disease at all, but that other mucosal inflammatory disorders, infectious or non-infectious, are the cause of the raised IgA levels in these patients. On the other hand it could be argued that only one klebsiella serotype was investigated in the study described here and that, furthermore, only the immune response against soluble bacterial antigens was tested. The strain used (obtained from Dr A F Geczy) has been found to contain antigens which may have an important role in HLA-B27-associated diseases.16 It has been shown to be a producer of 'modifying factor', a protein which is considered to be present on the lymphocyte membrane of most HLA-B27-positive ankylosing spondylitis patients and which was shown to interact with the lymphocytes of HLA-B27-positive healthy individuals. We may wonder whether any of this 'modifying factor' was present in the klebsiella culture filtrate used for coating the microcuvettes. Because for the ELISA technique the K43 strain was grown in a minimum essential medium, it is possible that certain bacterial antigens were not present in the culture filtrate, for it is known that the phenotypical expression of some bacterial antigens requires specific growth conditions.

A second possibility is that enteric infections with klebsiella organisms may be involved in the pathogenesis of the disease, but that this does not result in a stimulation of the klebsiella-specific immune response but rather in a polyclonal IgA stimulation.17 The hypothesis that a mucosal stimulation is involved is suggested by the fact that only the IgA levels but not the IgG levels are raised in the B27-positive uveitis patients.

Table 2  Total serum IgG and IgG anti-klebsiella in anterior uveitis*

<table>
<thead>
<tr>
<th></th>
<th>Anti-klebsiella IgG</th>
<th>Ratio</th>
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<tbody>
<tr>
<td>Controls</td>
<td>146±44</td>
<td>7.2±5.7</td>
</tr>
<tr>
<td>B27* AU+ AS+</td>
<td>166±46</td>
<td>5.4±2.7</td>
</tr>
<tr>
<td>B27* AU+ AS*</td>
<td>155±74</td>
<td>10.7±7.2‡</td>
</tr>
<tr>
<td>B27* AU+ AS*</td>
<td>143±43</td>
<td>8.1±6.0</td>
</tr>
</tbody>
</table>

*pTotal IgG levels are expressed as international units, whereas anti-klebsiella IgG is expressed in local ELISA units. Data represent the mean ± standard deviation. Ratios represent anti-klebsiella IgG divided by total IgG as calculated for each individual.

†p<0.001 (B27* AU+ AS+ vs. B27* AU+ AS*).
‡p<0.005 (B27* AU+ AS+ vs. B27* AU+ AS*).
Arguments which indicate that klebsiella organisms or cross-reactive bacteria do play a part in HLA-B27-associated uveitis stem from our finding that IgG levels against the klebsiella antigens were twice as high in the patients without ankylosing spondylitis than in those with ankylosing spondylitis. In conclusion, the findings reported here do not conclusively show an involvement of *Klebsiella pneumoniae* in the pathogenesis of HLA-B27-associated anterior uveitis, but they do suggest that factors resulting in an increased serum IgA play a part in this disease.

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References


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