Antibodies to a 15 kD Nuclear Antigen in Patients with Juvenile Chronic Arthritis and Uveitis

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Young girls with a pauciarticular onset of juvenile chronic arthritis and circulating antinuclear antibodies are at risk for chronic uveitis. The actual nuclear antigen for these antinuclear antibodies has not been defined. Conventional laboratory techniques, such as counter immunoelectrophoresis, have shown that antibodies to well defined “extractable nuclear antigens” (eg, RNP, Sm, SS-A, and SS-B) are not present in patients with juvenile chronic arthritis. Therefore, other, previously unknown nuclear antigens may be involved. Sera of 64 patients with juvenile chronic arthritis, including 22 patients with chronic anterior uveitis, were studied using the immunoblotting technique to characterize the nuclear antigens. Antinuclear antibodies were present in 12 (55%) of the 22 patients with uveitis, and only in six (14%) of the 42 patients without chronic anterior uveitis. With the immunoblotting technique, antibodies to a 15 kD nuclear antigen were found in 10 (45%) of the 22 patients with chronic anterior uveitis, whereas only two (5%) of the 42 patients without chronic anterior uveitis showed these antibodies \( P < 0.001 \). Only clearly visible and reproducible lines in the immunoblotting patterns were studied. This may provide a diagnostic tool for the early detection of uveitis and means for further pathogenetic studies. Invest Ophthalmol Vis Sci 33:1657-1660, 1992

Juvenile chronic arthritis (JCA) consists of a heterogeneous group of diseases, two of which are associated with potentially incapacitating ophthalmological complications. The major subgroups are systemic onset type, polyarticular onset type, and pauciarticular onset type.1

Pauciarticular disease is subdivided in two subgroups: type I, concerning mainly young girls with antinuclear antibodies (ANA) who are most at risk for developing chronic anterior uveitis; and type II (spondylitic type), concerning mainly boys in their teens, usually positive for HLA-B27, who sometimes develop acute anterior uveitis.

Chronic anterior uveitis is seen in 10–18% of patients with JCA.3,4 The onset of uveitis is insidious. The mean age of onset is 4 yr. Ocular complications already may have developed at first presentation of JCA or even before the first rheumatological symptoms. Eventually, band keratopathy and secondary cataract are seen in about 40% of patients, and secondary glaucoma is seen in about 20% of the children.3 If the children are seen at regular intervals by an ophthalmologist, the diagnosis can be made early. Promptly instituted therapy improves the visual outcome.5

ANA have been found in nearly 90% of patients with JCA and chronic anterior uveitis, whereas only about 25% of patients with JCA without chronic anterior uveitis have ANA.6,7 The strong association between ANA and chronic anterior uveitis in JCA suggests a role for these antibodies in the pathogenesis of the uveitis. In general, ANA are associated with generalized autoimmune diseases such as systemic lupus erythematosus.8,9,10 Autoantibodies against extractable nuclear antigens (ENA), such as antibodies to ribonucleoprotein (RNP), Sm antigen (Sm), Sjögren’s syndrome A antigen (SS-A), and Sjögren’s syndrome B antigen (SS-B), can be detected only in a few of the patients with JCA.11 Immunoblotting is a new technique that allows determination of a very large number of distinct ANA. In the present study, we used this method to search for previous unknown antinuclear antibodies that might be correlated with uveitis complicating JCA.

Materials and Methods

We collected 64 patients with JCA in two different ways. Of these children, 49 were seen in a pediatric
Table 1. Frequency of antinuclear antibodies (ANA) in juvenile chronic arthritis (JCA)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Liver cells</th>
<th>HEp-2 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCA without uveitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic onset</td>
<td>4 (25%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Polyarticular onset</td>
<td>11 (9%)</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>Pauciarticular onset</td>
<td>15 (13%)</td>
<td>4 (26%)</td>
</tr>
<tr>
<td>Juvenile ankylosing spondylitis</td>
<td>12 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>JCA with uveitis</td>
<td>22 (32%)</td>
<td>11 (55%)</td>
</tr>
<tr>
<td>Total</td>
<td>64 (17%)</td>
<td>17 (27%)</td>
</tr>
</tbody>
</table>

Of the 22 patients with chronic anterior uveitis, one had the systemic onset type, two had polyarticular onset type, and 19 had pauciarticular onset type. Of the 42 patients without chronic anterior uveitis, four had the systemic onset type, 11 had the polyarticular onset type, and 27 had a pauciarticular onset of disease.

Male to female ratio was 1 to 2.5 in the 42 children without chronic anterior uveitis and 1 to 2 in the 22 patients with chronic anterior uveitis. Ages of the children without chronic anterior uveitis ranged from 2-16 yr, with a mean of 11.5 yr. In patients with chronic anterior uveitis, ages ranged from 2-26 yr with a mean of 13 yr. The mean age of onset of arthritis was 6.8 yr in patients without chronic anterior uveitis. In patients with uveitis, mean age of onset of arthritis was 4.7 yr and mean age of onset of uveitis was 5.2 yr. Blood samples were obtained from 64 patients and plasma was stored at –20 C. Informed consent for the immunological tests was obtained from all parents.

ANAs were determined with the indirect immunofluorescence technique, using sera in a dilution of 1:10 on rat liver sections and in a dilution of 1:40 on HEp-2 cells.13 All sera were tested for anti-dsDNA antibodies on Crithidia luciliae at a 1:10 dilution.14 Determination of antibodies to ENA was performed by counter immunoelectrophoresis.15 IgM rheumatoid factor was measured with an ELISA technique using rabbit gamma globulin as an antigen and peroxidase-labelled anti-human IgM as a conjugate. Antibodies to centromere were determined according to the method described by Moroi et al.16

Immunoblotting of sera at a dilution of 1:50 with a nuclear antigen from HeLa cells was performed in duplicate on all sera according to Westgeest et al.17,18 The sera were coded and the results were read independently by three observers.

Results

All patients were tested for the presence of ANA on liver cell sections as well as on HEp-2 cells. Table 1 shows the results in patients with and without uveitis. The frequency of ANA using liver cells as substrate was 13% in patients without uveitis. When HEp-2 cells were used, this frequency was 20%. In patients with uveitis, these frequencies were 32% and 55%. Patients with juvenile ankylosing spondylitis showed no ANA on liver cells or on HEp-2 cells.

Antibodies to DNA and ENA were determined on all patients, regardless of circulating ANA in the indirect immunofluorescence test (Table 2). Weak antibodies against dsDNA were found in only one patient without uveitis. In three patients with JCA and uveitis, antibodies to DNA were found. No evidence of systemic disease other than JCA was found in any of these patients with antibodies to DNA.

One patient with uveitis had weakly positive SS-A antibodies. Clinically, this patient showed no evidence of SS-A-related diseases, such as Sjögren’s syn-
Fig. 1. Results of the immunoblotting experiments on sera of 40 of the 64 patients with juvenile chronic arthritis (nos. 11-51) are shown. The C pool consists of pooled sera of patients with systemic lupus erythematosus, mixed connective tissue disease, and CREST syndrome. Antibodies to the RNP antigen are represented by a line at 68 kD. Antibodies to the Sm antigen are represented by a line at 13 kD and a doublet at 28 kD. Antibodies to centromere are represented by a line at 16 kD. Antibodies to a 15-kD antigen can be seen in sera 15, 24, 27, 31, 32, and 46. Four of these six sera were from patients with juvenile chronic arthritis and chronic anterior uveitis.

Table 3. Absence of correlation between antinuclear antibodies (ANA) and the presence of antibodies to a 15-kD nuclear antigen

<table>
<thead>
<tr>
<th></th>
<th>ANA +</th>
<th>ANA -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-15-kD nuclear antigen present</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Anti-15-kD nuclear antigen absent</td>
<td>6</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>11</td>
<td>22</td>
</tr>
</tbody>
</table>

drome, systemic lupus erythematosus, or systemic sclerosis.

IgM rheumatoid factors were found in four patients with polyarticular JCA without uveitis and in two patients with polyarticular JCA with uveitis.

The results of the immunoblotting experiments are shown in Figure 1. The pool contains sera from patients with systemic lupus erythematosus, mixed connective tissue disease, and the CREST syndrome. Because reproducibility has been a problem with the immunoblotting technique, the sera were diluted to 1:50 or more, leaving only the clearest lines. Because of slight inequalities in running speed, the edges of the electrophoresis front are sometimes slightly curved, making exact measurement of the molecular weights difficult. Figure 1 shows that most antigens involved can be found between 28 and 70 kD. In the range below 20 kD, antibodies to a 15 kD antigen were found in sera from 12 patients. Ten of those 12 patients had chronic anterior uveitis. Only 2 of the 42 patients without chronic anterior uveitis showed such antibodies. The correlation between the presence of antibodies to the 15 kD antigen and uveitis in patients with JCA is statistically significant (chi squared = 13.1, P < 0.001). The presence of antibodies to this 15 kD antigen is virtually independent of the presence of ANA (Table 3). Determination of antibodies to centromeres, performed in the 12 patients with antibodies to the 15 kD antigen, were all negative.

**Discussion**

ANA are a common finding in patients with JCA and uveitis. Schaller et al\(^6\) and Kanski\(^7\) found frequencies of up to 90%. In the present study, 55% of the patients with JCA and uveitis showed ANA when determined with the indirect immunofluorescence technique on HEp-2 cells. This difference might be the result of a difference in clinical selection. However, a striking association between ANA and uveitis in patients with JCA remains.

This association stimulated a search for the nature of the ANA involved in the uveitis of JCA. Our study confirms that antibodies to ENA are not involved in
patients with JCA. This indicates that the high percentages of ANA are probably directed against other antigens, possibly detectable with the immunoblotting technique. Antibodies to dsDNA were found in one patient with polyarticular disease without uveitis and in three patients with uveitis, two with pauciarticular disease and one with systemic disease. This finding is consistent with the results of Alspaugh and Miller, who found antibodies to dsDNA in 3 out of 35 patients. 11

Until now, determination of antibodies to ENA in patients with JCA has been performed by only a few authors. 11,19,20 Alspaugh and Miller determined antibodies to RNP, Sm, SS-A, and SS-B by the Ouchterlony double diffusion method. 11 They found anti-RNP antibodies in 3% of the patients. Osborn et al. studied antibodies to RNP, Sm, SS-A, and SS-B by immunodiffusion. They found that none of the 20 patients tested positive. 19 In the present study, antibodies to RNP, Sm, SS-A, and SS-B were absent, except in one patient with JCA and uveitis, who was weakly positive to SS-A.

When testing sera of patients who have JCA with the immunoblotting technique, antibodies to a 15 kD nuclear antigen appeared in 10 of the 22 uveitis patients. These antibodies were seen in two of the 42 patients without uveitis. We also showed that antibodies to this 15 kD antigen also occur in uveitis without ANA positivity in other tests. We also have shown that antibodies to the 15 kD antigen are not identical to antibodies to centromeres. We also demonstrated that the artifacts described by Brinkman et al. 21 do not interfere with the detection of the antibodies to the 15 kD antigen. A further investigation into the nature of this 15 kD antigen is currently being performed.

Key words: juvenile chronic arthritis, uveitis, antinuclear antibodies, immunoblotting

References