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Letters to the Editor

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Immune complexes contain immunoglobulin A rheumatoid factor in serum and synovial fluid of patients with polyarticular juvenile rheumatoid arthritis

Sir. Immune complexes (IC) are believed to play a role in the pathogenesis of juvenile rheumatoid arthritis (JRA) [1]. Their prevalence varies from 39 to 79% depending on the method used for their detection [2]. Immunoglobulin M (IgM)- and IgG-containing IC activate the classical complement pathway, whereas IgA-containing IC activate an alternative complement pathway. Several groups, including ours, have shown complement activation through either or both pathways in patients with JRA [3, 4]. IgA rheumatoid factor (RF) has rarely been studied in IC isolated from patients with JRA. It may thus be interesting to determine if the IC in patients with JRA contain IgA RF.

Sera were obtained from 61 patients fulfilling the American College of Rheumatology criteria for JRA [5]. Synovial fluid specimens were available from seven of them. Sera from 25 healthy children were used as controls. Complement component C3 was measured by turbidimetry (Behring, Germany). Disease activity was defined based on previously described criteria [4].

Serum and synovial fluid specimens were tested for IC using a C3d-based enzyme-linked immunosorbent assay (ELISA) [6], using the mean + 2 s.d. (2.0 + 2 × 1.1 µg/ml) of the controls as the cut-off. From the specimens testing positive, IC were precipitated using polyethylene glycol (PEG) [7] and tested for IgA and IgM RF using ELISA [8, 9]. For the IgA RF assay, a known positive specimen from a patient with rheumatoid arthritis was used as the standard; absorbance readings for its doubling dilutions yielded a sigmoid curve and the concentration at the beginning of the upper plateau was assigned a value of 100 arbitrary units (AU/ml) of IgA RF. For IgM RF, a WHO standard (WHO, Geneva) was used and concentrations were expressed as IU/ml. Wilcoxon’s rank sum test, Fisher’s exact test and the $\chi^2$ test were used for analysis.

Of the 61 patients (42 male; median age 11 yr, median disease duration 3 yr) studied, 25 (15 male), 17 (14 male) and 19 (13 male) had polyarticular, pauciarticular and systemic onset types of JRA, respectively. Eight patients had RF detectable by latex agglutination. Serum C3 levels (mean ± s.d.) were: polyarticular type (180.7 ± 79.3 mg/dl), pauciarticular type (198.8 ± 88.0 mg/dl) and systemic onset type (232 ± 110.4 mg/dl).

In 33 of the 61 (54%), the sera had detectable IC (control 3/25; $P < 0.0001$). The prevalence of IC in polyarticular, pauciarticular and systemic onset types was 64% (16/25), 59% (10/17) and 37% (7/19), respectively. The prevalence of IC was higher in patients with active disease (30/49 vs 3/12 in inactive disease; $P < 0.05$).

Of the 33 patients with IC, IgA RF and IgM RF were detected in PEG-precipitated IC in nine and 12 patients, respectively. Four patients had both IgA RF and IgM RF. IgA RF was detected more often among patients with polyarticular onset type (8/16) than in those with pauciarticular onset (0/10; $P < 0.001$) and systemic onset (1/7; $P = $ns) types. The prevalence of IgM RF was similar in the three onset groups (7/16, 2/10 and 3/7, respectively).

Of the seven synovial fluid specimens (all from patients with IC in the serum; with IgM RF and IgA RF in two and four patients, respectively), six contained IC; of these, none contained IgM RF, whereas four had IgA RF. Synovial fluid C3 levels were below 50% of the simultaneous serum C3 concentration (Table 1).

**Table 1. Characteristics of simultaneous sera and synovial fluid specimens in patients with JRA.**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Onset type of disease</th>
<th>Serum C3 (mg/dl)</th>
<th>IC (µg/ml)</th>
<th>IgM RF (IU/ml)</th>
<th>IgA RF (AU/ml)</th>
<th>Synovial fluid C3 (mg/dl)</th>
<th>IC (µg/ml)</th>
<th>IgM RF (IU/ml)</th>
<th>IgA RF (AU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Polyarticular</td>
<td>110</td>
<td>16.5</td>
<td>1.56</td>
<td>8.30</td>
<td>35.7</td>
<td>6.5</td>
<td>1.56</td>
<td>2.6</td>
</tr>
<tr>
<td>2</td>
<td>Polyarticular</td>
<td>171</td>
<td>8.5</td>
<td>1.56</td>
<td>8.00</td>
<td>101</td>
<td>9.0</td>
<td>1.56</td>
<td>3.6</td>
</tr>
<tr>
<td>3</td>
<td>Systemic onset</td>
<td>175</td>
<td>7.5</td>
<td>2.00</td>
<td>2.60</td>
<td>Not done</td>
<td>31.0</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>4</td>
<td>Polyarticular</td>
<td>118</td>
<td>11.0</td>
<td>1.56</td>
<td>1.56</td>
<td>51.2</td>
<td>2.5</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>5</td>
<td>Polyarticular</td>
<td>118</td>
<td>7.0</td>
<td>3.82</td>
<td>1.56</td>
<td>49.5</td>
<td>8.0</td>
<td>1.56</td>
<td>4.16</td>
</tr>
<tr>
<td>6</td>
<td>Polyarticular</td>
<td>137</td>
<td>14.5</td>
<td>1.56</td>
<td>14.30</td>
<td>&lt;30</td>
<td>32.5</td>
<td>1.56</td>
<td>7.95</td>
</tr>
<tr>
<td>7</td>
<td>Polyarticular</td>
<td>136</td>
<td>15.0</td>
<td>1.56</td>
<td>1.56</td>
<td>64.3</td>
<td>32.5</td>
<td>1.56</td>
<td>1.56</td>
</tr>
</tbody>
</table>

Values of IgM RF and IgA RF exceeding 1.56 IU/ml and 1.56 AU/ml are abnormal.
The prevalence of CIC in our JRA patients was similar to that observed in previous studies [1–3]. The association of the presence of IC with disease activity supports the role of IC in the pathogenesis of JRA. Our observations of the presence of IC in synovial fluid, a high concordance of serum and synovial fluid IC and a higher concentration in synovial fluid than in serum, suggest that IC may have a role in causing synovitis. Also, the low C3 levels in synovial fluid may reflect local complement activation by these IC.

The presence of IgA RF both in serum and synovial fluid IC suggests a pathogenetic role for IgA RF IC in patients with the polyarticular type of JRA. Their role is further supported by better correlation of circulating IC levels with levels of factor Bb [3] and the presence of IgA RF in patients with severe polyarticular JRA [9].

What is the origin of IgA RF in serum and synovial fluid of patients with JRA? Serum IgA RF levels have recently been shown to be increased in patients with Lyme arthritis [10], an infectious disease; this raises an interesting possibility that infectious organisms may be involved in the generation of IgA RF in patients with JRA. Another possible source could be the B cells which form ectopic lymphoid tissue in the synovium.

Our data have some limitations. First, the PEG precipitation method used may precipitate some other aggregates and Ig monomers. Second, IgA RF was measured in PEG precipitate, which includes not only C3d-bound IC but total IC. Third, only a few synovial fluid specimens were studied.

In conclusion, our data suggest that there may be a pathogenetic role for IC that contain IgA RF in patients with polyarticular JRA. Further studies are needed to elucidate the mechanisms involved, like alternative complement pathway activation or cytokine release.

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