Evidence for activation of the alternate complement pathway in patients with juvenile rheumatoid arthritis

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Abstract

Objective. Complement activation has been shown to occur in patients with juvenile rheumatoid arthritis (JRA). Since the two pathways of complement are activated by different stimuli (the alternate pathway by microbial products and IgA, and the classical pathway by immune complexes), we decided to study the relative contribution of the two pathways of complement activation in patients with JRA.

Methods. In 56 patients with JRA, plasma levels of C3 and C4 were measured by turbidimetric assays, and those of C4d, factor Bb and sC5-9 complex by solid-phase enzyme immunoassays. Levels beyond the mean ± 2 s.d. of normal were considered abnormal.

Results. Plasma C3 and C4 levels were decreased in one patient each. The C4d values were increased in 17 patients, whereas levels of factor Bb were elevated in 42 patients and levels of sC5-9 complex were elevated in 51 patients. The values of factor Bb and sC5-9 had a linear correlation (r = 0.75), but there was no significant correlation between C4d and sC5-9 levels (r = 0.36).

Conclusion. Complement activation in JRA is initiated predominantly by the alternate pathway and culminates in the formation of terminal membrane attack complex.

Key words: Juvenile chronic arthritis, Immune complexes, Pathogenesis.
Patients and methods

Fifty-six patients with JRA satisfying the American College of Rheumatology (ACR) criteria [7], and seen at our clinic between April 1996 and October 1997, were enrolled in the study. Data regarding the type of disease pattern, presence of disease activity (duration of early morning stiffness, number of active joints, presence of systemic manifestations like fever, rash, hepatosplenomegaly, lymphadenopathy) and clinical course were recorded. In addition, current medications were also recorded. The erythrocyte sedimentation rate (ESR) and concentration of haemoglobin and C-reactive protein (CRP) were measured as laboratory parameters of disease activity. Active disease was defined as the presence of systemic manifestations and/or of active synovitis (tenderness/synovial effusion/early morning stiffness > 30 min) in at least three joints (for polyarticular disease) and at least one joint (for pauciarticular disease) in association with elevated CRP (>1.2 mg/dl) or ESR (>25 mm fall in first hour). All patients were carefully screened for any infection in the preceding 4 weeks and, if there was any doubt, the patient was excluded from the study. In three patients three samples each, and in seven patients two samples each, were collected at different time points. Plasma samples from 18 sex-matched young healthy controls, including eight children, were used as controls.

For complement studies, blood was collected in EDTA and transported on ice to the laboratory. Plasma was separated and then stored at -70°C until analysis. Levels of C3 and C4 were measured by a turbidimetric method using anti-C3c and anti-C4 antibodies (Behring, Germany). Concentrations of complement degradation products (C4d, factor Bb and sC5-9 complex) were measured by enzyme-linked immunosorbent assays (EIA) (Quidel Corporation, San Diego, CA, USA).

The C4d level was used as a measure of activation of the classical pathway, whereas factor Bb levels reflected activation of the alternate pathway. The sC5-9 levels are a measure of the amount of the TAC generated as a result of activation of the common pathway by either the classical or alternate pathway and is thus a sensitive indicator of overall complement activation.

In brief, test plasma specimens in appropriate dilution were added to EIA wells coated with monoclonal antibody to the respective complement product and incubated for 1 h. After washing, a second antibody conjugated with horseradish peroxidase was added. This was followed by the addition of tetra methyl benzidine (TMB) substrate; the reaction was stopped after 15 min and the absorbance read at 450 nm. For each analyte, concentrations in the test samples were read from a standard plot between absorbance and concentration of a set of standards. Values above the mean ± 2 s.d. of normal healthy controls were taken as abnormal.

Statistical analysis

Pearson’s correlation coefficient was used to study the relationship of various continuous variables with one another. Kruskal–Wallis one-way ANOVA was used for detecting differences between different subtypes. An alpha error of <0.05 was considered significant.

Results

The mean age of patients was 13.0 ± 5.0 yr (range 4.0–18.0 yr) and the mean duration of disease was 3.5 ± 1.5 yr (range 0.3–8.0 yr). Of the 56 patients studied, 22 had polyarticular onset, 21 had systemic onset and 13 had pauciarticular onset type of disease. The disease was active in 51 patients and inactive in five patients. Fifty-four patients were receiving non-steroidal anti-inflammatory drugs and 14 were also receiving weekly oral low-dose methotrexate.

Of the 56 patients, C3 and C4 levels were decreased in one patient each. The levels of the complement degradation products, C4d, factor Bb and sC5-9 complex, were higher in patients as compared with controls (P < 0.01). The C4d levels were increased in only 17 patients, whereas levels of factor Bb were elevated in 44 patients and those of sC5-9 complex were increased in 51 patients. There was a trend towards higher levels of C4d, Bb and sC5-9 in polyarticular and systemic-onset subsets of JRA, but it was not statistically significant, probably due to the small number of patients in each group (Table 1).

Among the patients with elevated sC5-9, only three had elevated C4d alone, 28 had elevation of factor Bb alone, whereas in 14 both C4d and factor Bb were elevated. In six patients with elevated sC5-9, both C4d and factor Bb were normal. The value of Bb and sC5-9 had a linear correlation (r = 0.747, P < 0.01) (Fig. 1), while there was no significant correlation between C4d and sC5-9 levels (r = 0.36, P > 0.05). In addition, there was no significant correlation (r = 0.167) between C4d and factor Bb.

In 10 patients, for whom two or three serial samples were tested, there was a direct relationship between the levels of factor Bb and sC5-9; a rise in one was associated with a rise in the other (Fig. 2), whereas the levels of C4d had no such relationship with sC5-9 levels.

Discussion

Our data show that sC5-9 levels are elevated in a majority of patients with JRA. This indicates that the complement pathway is activated in these patients, resulting in the formation of membrane TAC. Activation of the complement pathway has previously been shown to occur [1, 3, 5, 6]; however, the formation of membrane TAC has been rarely reported. Our data may thus provide more conclusive evidence for the pathogenetic significance of complement pathway activation in JRA. The exact site of membrane TAC generation is not known and could be synovium, skin (in systemic onset) or spleen.

Complement activation can occur by the classical or the alternate pathway, or by a combination of the two. Our data suggest that the alternate pathway is the major
Table 1. Median [range] levels of complement components in patients with different types of JRA

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (18)</th>
<th>Pauciarticular (13)</th>
<th>Polyarticular (22)</th>
<th>Systemic onset (21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 (mg/dl)</td>
<td>92.0 [60.2–110.5]</td>
<td>114 [65.5–266.0]</td>
<td>162 [47.1–344.0]</td>
<td>188 [91.4–450.0]</td>
</tr>
<tr>
<td>Factor Bb (µg/ml)</td>
<td>0 [0–2.4]</td>
<td>2.9 [1.5–16.5]</td>
<td>3.2 [1.5–6.9]</td>
<td>3.4 [1.7–40.8]</td>
</tr>
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</table>

contributor in patients with JRA, with a minor contribution by the classical pathway.

Activation of the classical pathway has been reported to occur in polyarticular, systemic-onset disease [1, 5] and pauciarticular-onset disease [3, 5]. Higher levels of C4d in systemic-onset disease could suggest that in this variant more IgG- and IgM-containing immune complexes are generated which cause classical pathway activation.

Activation of the alternate pathway found by us was also present in the study reported by Jarvis et al. [5], but in that study patients with systemic-onset disease were not included. Thus, our study is the first report of alternate pathway activation in systemic-onset disease. The presence of alternate pathway activation in three-quarters of patients suggests that it may have a role in disease pathogenesis. In a recent study [6], levels of factor Bb were found to correlate better with the level of circulating immune complexes than levels of C4d, implying that immune complexes were activating the alternate pathway.

Alternate pathway activation can occur by immune complexes bearing IgA rheumatoid factor (RF). Indeed, high-molecular-weight IgA RF-containing immune complexes have been described in children with polyarticular disease [8]. The alternate pathway is also the major mechanism of solubilization of large preformed aggregates [9], and there may be some immune complexes preformed in the tissues in patients with JRA which can activate the alternate pathway when they reach the circulation. In addition, bacterial products can activate the alternate pathway. Analysis of immune complexes from patients with JRA have revealed novel 40 and 60 kDa bands which are resistant to DNase, RNase and proteases, suggesting that these may be lipopolysaccharide-enterobacterial protein complexes, glycosaminoglycans or some as yet unknown compounds [10]. In contrast, in 1990, Olds and Miller [3] failed to show the presence of antibodies to streptococcal cell wall peptidoglycan–polysaccharide polymers in the immune complexes isolated from patients with JRA, indirectly showing that these antigens are not present in immune complexes of patients with JRA. Rarely, a low level of C3b generated from activation of the classical pathway can perpetuate activation of the alternate pathway.

In addition, there may be some inhibitors of classical pathway activation like IgM RF, which is found in JRA and may inhibit the covalent binding of C4b to IgG in the immune complexes and thus prevent the classical pathway activation [11].

Fig. 1. Scatterplot for plasma Bb and plasma sC5-9 complex. The Pearson’s correlation coefficient is 0.75 ($P < 0.001$).

Fig. 2. Line diagram showing serial values of plasma Bb and plasma sC5-9 complex in three patients. The solid line represents factor Bb levels (µg/ml), while the dashed line (---) represents sC5-9 complex levels (µg/ml). On the x-axis, the time in months represents time since inclusion in the study.
Thus, complement activation occurs in the majority and these complement degradation products, by inducing inflammation [12], could contribute to synovitis. Since JRA is a heterogeneous disease and the different subtypes may have a different pathogenesis, and we had a small number of patients of each subtype, our results need to be validated in a larger sample. In addition, analysis of immune complexes in sera of patients with JRA for the presence of IgA RF, and the putative microbial products, may help identify the triggers for complement activation.

Acknowledgement

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References