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Immunologic Studies of Juvenile Rheumatoid Arthritis

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Antigammaglobulins of the IgG, IgA and IgM classes, and whole hemolytic complement and complement components were measured in the sera and synovial fluid of patients with juvenile rheumatoid arthritis. Two groups of patients were distinguishable using these measurements. A group of patients with chronic active disease had elevated levels of IgG, IgA and IgM antigammaglobulins and relative depressions of complement and complement components in sera and synovial fluid. Another group of patients with acute self-limited disease had elevated levels of IgG and IgA antigammaglobulins and serum complement and negative latex fixation tests in association with disease activity. These values returned to normal with clinical remissions.

While there is now ample evidence to implicate rheumatoid factors and complement in the inflammatory synovitis of adult rheumatoid arthritis (RA), little is known about the immunopathology of juvenile rheumatoid arthritis (JRA). One of the striking abnormalities in patients with RA has been the high frequency, 60–90%, with which rheumatoid factors (RF) have been found (1–4). Synovial fluid complement levels are often depressed (5–7), and serum levels may also be diminished (8, 9) in RA. In contrast, rheumatoid factors have been demonstrated in only 12–22% of sera from patients with JRA (10–13). Complement measurements in these patients have received scant attention in other reviews of the immunology of adult RA (14).

The purpose of the present study was to determine whether or not there were significant differences in the immunopathology of JRA and RA. A number of immunologic parameters were evaluated in a large series of patients with JRA. Measurements were made of IgG, IgA and IgM antigammaglobulins, "cold reactive" rheumatoid factors, complement and complement components in sera and synovial fluids. The results of these studies were compared with

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those found in patients with RA and related to a number of clinical and laboratory features of JRA.

MATERIALS AND METHODS

Patients

Sera from all 148 patients with definite JRA (15), seen at the Robert Breck Brigham Hospital between 1967 and 1970, were studied. The patients had a mean age of 15 years (range: 2–43). Patients with JRA who had positive serum latex fixation tests for RF at 37°C will hereafter be designated as RF (+) and patients with a negative test at 37°C will be referred to as RF (−). Sera from 50 normal individuals (mean age: 22 years, range: 15–29) and from 17 patients with cystic fibrosis* (mean age: 10 years, range: 4–15) were used as controls.

Clinical Studies

The patients' sex and age at the time of the study as well as at the onset of disease were recorded. Joint involvement was characterized as monoarticular or polyarticular. The presence of fever, rash, lymphadenopathy, hepatomegaly, splenomegaly, uveitis, pericarditis or subcutaneous nodules was noted. The stage of disease and functional class (16) were assessed. The disease was considered to be active when local articular swelling and an elevated sedimentation index were present. Many of the patients underwent serial observations over periods of several years. Patients were considered to have an unfavorable course if they had persistent active arthritis, poor or worsening functional class or progressive joint damage. A favorable course was characterized by lessening of disease activity, no worsening and frequently improvement in the functional class. Patients with diagnoses of rheumatic fever, systemic lupus erythematosus, polymyositis, ankylosing spondylitis, progressive systemic sclerosis, inflammatory bowel disease, hypertrophic osteoarthropathy or chronic liver disease were excluded from this study.

Laboratory Studies

The following laboratory studies were performed: complete blood count, blood sedimentation index (17), serum protein electrophoresis, LE cell preparation, latex fixation test (LFT) (18) and antinuclear antibody test (19). In those patients with antinuclear antibodies, tests for precipitating antibodies to calf thymus deoxyribonucleic acid, heat denatured DNA, calf thymus soluble extract (CTN), rabbit liver ribosomes (20) and ribonucleic acid (21) were performed.

Synovial fluid specimens were available from 16 patients with JRA. They were analyzed for cell count and differential, protein and glucose levels, mucin clot formation (22), phagosomes (23), as well as by the special immunologic investigations which will be described.

Immunologic Studies

Antigammaglobulins of the IgG, IgA and IgM classes were measured in the sera of 92 patients and SF of 16 patients with JRA. The assay was performed by the adsorption of samples to, and the elution from, insoluble human IgG as already reported from this laboratory (24). Briefly, human IgG was insolubilized by cross-linking with glutaraldehyde, according to the method of Avrameas and Ternynck (25). Aliquots of serum or SF were incubated with the insoluble IgG. After the immunoadsorbent was washed, antigammaglobulins were eluted with 0.1M glycine-HCL pH 2.8 buffer. Eluates were brought to neutral pH, filtered and concentrated to the original sample volume. Over 98% of the protein content were immunoglobins. Antigammaglobulins of the IgG, IgA and IgM classes were then assayed by radial immunodiffusion (26). The immunoadsorbent was stable under these conditions and no IgG was released when incubated with either acid or neutral buffers. Antigammaglobulins of each immunoglobulin class were isolated and separated from one another by chromatography on columns of Sephadex G200, diethylaminoethyl cellulose and carboxymethyl cellulose. Isolated γG, γA and γM antigammaglobulins each possessed latex agglutinating activity.

"Cold reactive" rheumatoid factor (27) was determined in sera from normal individuals, patients with JRA, cystic fibrosis, osteoarthritis, RF (−) RA and systemic lupus erythematosus. This determination was performed by a modification of the latex fixation test of Singer and Plotz (18). Serum was incubated with human γ-globulin-coated latex particles at 4°C for 18 hours, and then the degree of agglutination was read in each tube. Studies were done both with and without inactivating the sera at 56°C for 30 minutes. Titers greater than 1:20 were considered positive. A standard LFT (18) was carried out concurrently.

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Total hemolytic complement determinations (CH_{50}) were made according to the method of Kent and Fife (28). The normal range for serum in this laboratory is 200 ± 50 μ/ml. Measurements of Clq (29) C4, and C3 (30) protein levels were carried out by radial immunodiffusion.

Data Analysis

Standard statistical methods were employed for *t* tests, chi square tests and for calculating *P* values.

RESULTS

Serum Antigammaglobulin Levels

Levels of serum IgM antigammaglobulins are presented in Figure 1. Each point in the figure represents a determination from an individual patient's serum. The solid line denotes the mean value and shaded area indicates the 95% confidence limit for the mean. With the exception of one normal individual, IgM antigammaglobulins were only detected in RF(+) JRA patients. The mean value for these 15 patients was 100 ± 30 μg/ml (range: 26–270 μg/ml).

Serum IgG antigammaglobulins are similarly shown in Figure 2. They were detected in all individuals studied. Mean levels were 98 ± 18 μg/ml (range: 16–300 μg/ml) for normal individuals, 69 ± 22 μg/ml (range: 30–126 μg/ml) for patients with cystic fibrosis, 140 ± 20 μg/ml (range: 20–378 μg/ml) for RF(−) patients with JRA, and 182 ± 44 μg/ml (range: 80–369 μg/ml) for patients with RF(+) JRA. The mean level for all patients with JRA, 157 ± 18 μg/ml, was significantly greater than the mean levels for either the group of normal individuals or patients with cystic fibrosis ($P < 0.05$). Levels of IgG antigammaglobulins were greater in patients with RF(+) JRA than among other groups of patients.

Serum IgA antigammaglobulin levels are depicted in Figure 3. They were detected in 76% of normal individuals with a mean level of 52 ± 10 μg/ml (range: <20–132 μg/ml), in 23% of patients with cystic fibrosis with a mean level of 24 ± 8 μg/ml (range: <20–58 μg/ml), 77% of patients

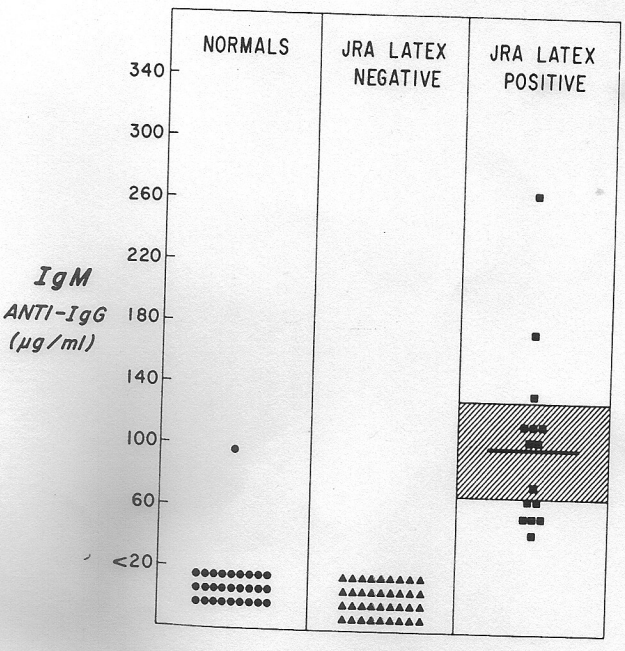

Fig 1. Serum IgM antigammaglobulin levels in normal individuals are represented by closed circles. Levels in patients with latex negative juvenile rheumatoid arthritis (JRA) are shown by triangles. Levels in patients with latex positive JRA are indicated by squares. Solid lines denote the mean value for each group and the shaded areas indicate the 95% confidence limit for the means.

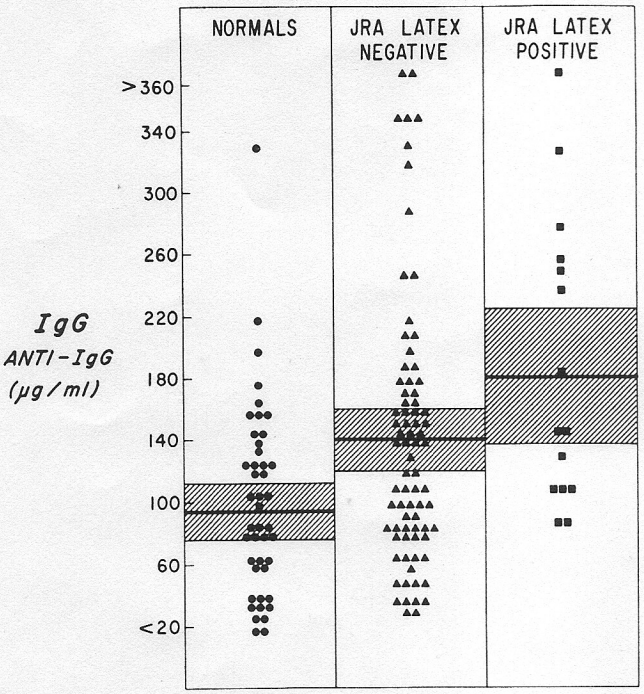

Fig 2. Serum IgG antigammaglobulin levels in normal individuals are represented by closed circles. Levels in patients with latex negative juvenile rheumatoid arthritis (JRA) are shown by triangles. Levels in patients with latex positive JRA are indicated by squares. Solid lines denote the mean value for each group and the shaded areas indicate the 95% confidence limit for the means.

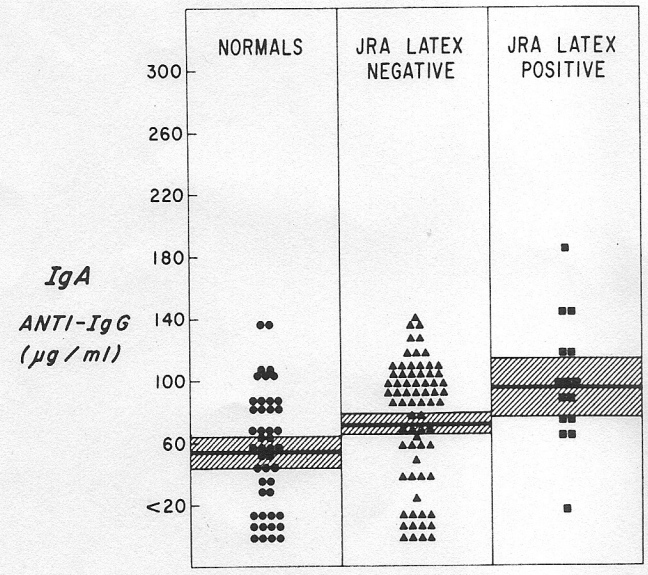

Fig 3. Serum IgA antigammaglobulin levels in normal individuals are represented by closed circles. Levels in patients with latex negative juvenile rheumatoid arthritis (JRA) are shown by triangles. Levels in patients with latex positive JRA are indicated by squares. Solid lines denote the mean value for each group and the shaded areas indicate the 95% confidence limit for the means.

with RF(−) JRA with a mean level of 70 ± 8 μg/ml (range: <20–160 μg/ml), and in all patients with RF(+) JRA with a mean level of 98 ± 18 μg/ml (range: 57–200 μg/ml). The mean level for all patients with JRA, 74 ± 8 μg/ml, was significantly greater than the mean levels for either the group of normal individuals or patients with cys-

tic fibrosis ($P < 0.05$). Levels of IgA antigammaglobulins were greater in patients with RF(+) JRA than among other groups of patients.

High levels of serum IgG, IgA and IgM antigammaglobulins usually occurred together. An elevation of serum antigammaglobulins of any single immunoglobulin class was generally associated with high serum levels of antigammaglobulins of the other two immunoglobulin classes studied.

Fifteen patients with elevated levels of serum IgM antigammaglobulins were identified and compared to the remaining 77 patients with JRA who had no detectable serum IgM antigammaglobulin (Table 1). These 15 patients were characterized by markedly elevated levels of serum IgG and IgA antigammaglobulins, relative depression of serum complement levels, greater incidence of antinuclear factor, late onset of disease, polyarticular joint involvement, poor anatomic stage and functional class, frequent radiologic evidence of joint destruction, frequent subcutaneous nodules, an unfavorable course, and a positive LFT ($P < 0.05$).

Table 1. Significance of IgM Antigammaglobulins in JRA

	Present (N = 15)	Undetectable (N = 77)
Age of onset of disease (yr)	12	6
Polyarticular involvement (%)	100	80
Stage and functional Class III–IV (%)	80	30
Radiologic evidence of joint disease (%)	90	37
Subcutaneous nodules (%)	20	2
Unfavorable course (%)	70*	16
Elevation of serum IgG and IgA antigammaglobulin levels	↑↑	↑
Serum CH_{50}	181 μ/ml*	209 μ/ml
Antinuclear antibodies (%)	47*	5

* ($P < 0.05$)

Serum antigammaglobulin levels in patients with JRA with inactive and active disease are shown in Table 2. Among 36 patients with active disease, 12 were RF(+) and 24 RF(−). Serum IgG, IgA, and IgM antigammaglobulin levels were similar in RF(+) patients with active or inactive disease. By contrast, significant elevations of serum IgG and IgA antigammaglobulins were observed in RF(−) patients with active disease as compared to RF(−) patients with inactive disease ($P < 0.05$).

Cold reactive serum antigammaglobulin determinations are summarized in Table 3. When the LFT was carried out without inactivating the sera, cold reactive antigammaglobulins were found in many of the sera tested. When the LFT at 4°C was performed with heat inactivated sera, cold reactive antigammaglobulins were detected in all the sera that reacted at 37°C. In addition, they were detected in 17 of the 77 (22%) sera of patients with JRA who had a negative LFT at 37°C. These cold reactant antigammaglobulins were found infrequently in the other patients studied ($P < 0.05$). These 17 JRA patients with cold reactive antigammaglobulins had an elevated IgG (mean: 152 ± 32 μg/ml) antigammaglobulin level, a greater frequency of X-ray evidence of joint destruction and a

Table 2. Antigammaglobulin Levels in JRA

	No. of patients	Immunoglobulins IgG	IgA	IgM (μg/ml)
Active				
Latex (+)	12	171	108	94
Latex (−)	24	161	94	<20
Remission				
Latex (+)	3	170	86	58
Latex (−)	53	129	63	<20

Table 3. Cold Reactive Antigammaglobulins in JRA

Groups	No. of patients	Latex Fixation (no. positive) 37°C	4°C
Normals	50	0	0
JRA			
Latex (+)	15	15	15
Latex (—)	77	0	17*
Cystic fibrosis	17	0	0
Osteoarthritis	45	0	0
Systemic lupus erythematosus	45	2	2
Rheumatoid arthritis latex (—)	45	0	4

* ($P < 0.05$)

more unfavorable course ($P < 0.01$) than those JRA patients with nondetectable cold reactive antigammaglobulin.

Serum Complement Levels

Serum complement levels were measured in 148 patients with JRA and are shown in Table 4. Values in these patients ranged from 120–390 μ/ml with a mean value of 206 ± 8 μ/ml. Forty-three patients with JRA had determinations performed when initially seen with active disease, and again 2 to 4 years later when the disease was quiescent. The mean CH_{50} level for these patients was 267 ± 15 μ/ml (range: 210–390 μ/ml) during the time of active disease, and 198 ± 15 μ/ml (range: 155–250 μ/ml) when in clinical remission. This difference was statistically significant ($P < 0.01$). Eighteen RF(+) patients with JRA had a mean CH_{50} level of 185 ± 15 μ/ml (range: 135–210 μ/ml) while 130 RF(—) patients with JRA had a mean serum CH_{50} level of 208 ± 8 μ/ml (range: 130–390 μ/ml) ($P = 0.05$).

Serum C1q, C3 and C4 protein levels were determined in the 16 patients with JRA in whom SF specimens were available. Thirteen of these patients were RF(—) and three were RF(+). Mean serum C1q levels were 23.3 μg/N/ml for RF(—) and 26.6 μg/N/ml for RF(+) patients. Mean serum C3 levels were 175 mg% for RF(—) and 193 mg% for RF(+) patients. Mean serum C4 levels were 543 μg/ml for RF(—) and 328 μg/ml for RF(+) patients. Despite the small number of patients studied in this manner, the difference of mean serum C4 protein levels in these groups was significant ($P < 0.05$).

Table 4. Serum Complement (CH_{50}) Levels In 148 Patients With JRA

	No. of patients	Mean CH_{50} Levels ± 2 SE (μ/ml)
Active	44	267 ± 18*
Remission	44	197 ± 15
Latex (+)	18	185 ± 15†
Latex (—)	130	208 ± 8

* $P < 0.01$
† $P = 0.05$

Synovial Fluid Antigammaglobulin Levels

Levels of antigammaglobulins in the SF studied are summarized in Table 5. IgG antigammaglobulins were detected in all SF specimens. The levels for patients with RF(—) JRA (mean 170 μg/ml) and RF(+) JRA (mean 180 μg/ml) were similar.

Table 5. Synovial Fluid Antigammaglobulin and Complement Levels in JRA

Groups	No. of patients	Immunoglobulins (μg/ml*) IgG	IgA	IgM	CH_{50} (μ/ml*)
Latex (+)	3	180	91	104	33
Latex (—)	13	171	85	<20	99

* mean values

IgA antigammaglobulins were found in 9 of 13 patients with RF(−) JRA with a mean level of 85 μg/ml, and in 2 of 3 patients with RF(+) JRA with a mean level of 91 μg/ml. These levels were similar.

IgM antigammaglobulins were detected only in SF from patients with RF(+) JRA. The mean value for these patients was 104 μg/ml.

Although no differences were observed between these small numbers of patients with JRA with respect to SF IgG and IgA antigammaglobulins, their levels were similar to those reported previously for patients with RA and are similarly elevated when compared to patients with osteoarthritis (24).

Synovial Fluid Complement Levels

Synovial fluid CH_{50} (Table 4) and C1q, C3 and C4 protein levels (Fig 4) were measured in the 13 RF(−) and three RF(+) SF available. The mean level of SF CH_{50} for RF(−) patients was 99 μ/ml (range: 20–246 μ/ml) and for RF(+) patients was 33 μ/ml (range: 13–70 μ/ml) ($P < 0.05$).

The mean C1q level for RF(−) patients was 10.3 μg/N/ml (range: 7–20 μg/N/ml) and for RF(+) patients was 8.3 μg/N/ml (range: 7–11 μg/N/ml).

The mean C3 level for RF(−) patients was 79 mg% (range: 20–162 mg%) and for RF(+) patients was 40 mg% (range: 37–55 mg%), ($P = 0.05$).

The mean C4 level for RF(−) patients was 202 μg/ml (range: 60–630 μg/ml) and for RF(+) patients was 5 μg/ml (range: 0–10 μg/ml), ($P < 0.01$).

A trend toward an inverse correlation of SF CH_{50} and SF IgG antigammaglobulin levels was observed. There were too few patients with detectable IgM antigammaglobulins in the SF to meaningfully relate this measurement to SF CH_{50}.

JRA patients with depressed CH_{50}, C4 and C3 levels in the SF tended to have severe disease, unremitting synovitis, and an unfavorable course.

Antinuclear Antibodies

One hundred forty-eight serum samples from patients with JRA were examined for antinuclear antibodies (ANA). A test was considered positive only when unequivocal fluorescence was visible at a serum dilution of 1:10. Twenty-two patients (14%) had ANA in their serum. Of the 22 patients, 12 were RF(−) and 10 were RF(+). Thus ANA were found in the sera of 12 of 130 (9%) RF(−) JRA patients and 10 of 18 (56%) patients with RF(+) JRA ($P < 0.01$). Sixteen synovial fluids (SF) were also examined for ANA. One of 13 RF(−) SF

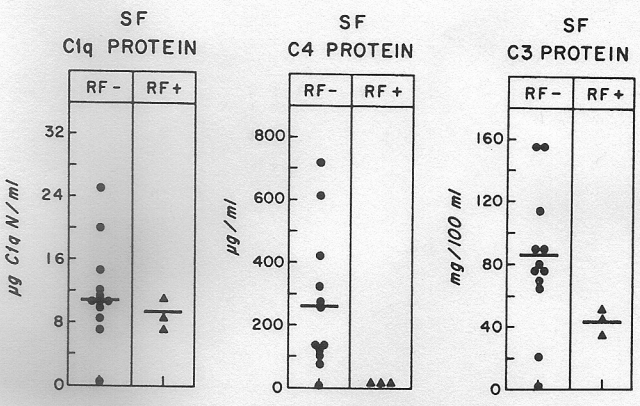

Fig 4. Synovial fluid C1q, C4 and C3 protein levels in patients with latex negative juvenile rheumatoid arthritis (JRA) are indicated by closed circles. Levels in patients with latex positive JRA are represented by triangles. Solid lines denote the mean values for each group.

had ANA (as did the patient's serum). Three of three RF(+) SF had ANA (as did the patients' serum).

Immunofluorescent Studies

Immunofluorescent staining was performed on SF leukocytes from five patients with RF(−) JRA. Phagosomal intracellular inclusions of IgG were seen in two patients, IgA in one patient, IgM in two patients, C1q in one patient, β1E and β1C in two patients and human serum albumin in none. No patients had phagosomal staining for fluoresceinated aggregated IgG. Intracellular deposition of immunoglobulins or complement could not clearly be related to SF antigammaglobulin or complement levels in these five patients.

DISCUSSION

Rheumatoid factors, as detected by agglutination methods, have been associated and implicated with the severe inflammatory changes and clinical manifestations of adult RA. In addition to these IgM antigammaglobulins, increased levels of IgA and IgG antigammaglobulins have been detected in the serum and synovial fluids of patients with RA (24). Elevated levels of these antigammaglobulins were each associated with clinical findings of severe disease and particularly related to diminished serum and synovial fluid complement levels. These observations indicated that rheumatoid factor production is distributed among all immunoglobulin classes, thus broadening the concept of an exaggerated immune response in these patients.

Rheumatoid factors, when measured by the latex fixation or sensitized sheep cell agglutination tests occur infrequently in patients with JRA (10–13). These rheumatoid factors, generally assumed to be 19S IgM antibodies (31), have been associated with active and late onset of the disease (10, 13), poor functional class, elevated sedimentation rate and hip involvement (11), the presence of subcutaneous nodules (10, 13) and small joint disease (13). Torrigiani *et al* (32) have found elevated levels of IgG, but not IgA or IgM antigammaglobulins in the sera of patients with JRA. Levels were elevated particularly in those patients with active disease. In the present study, levels of IgG, IgA, and IgM antigammaglobulins were found to be higher in the sera of patients with JRA than in normal individuals or patients with cystic fibrosis or osteoarthritis (24). Patients with elevated levels of not only IgM, but also IgG and IgA antigammaglobulins had those clinical parameters known to be related to a positive latex fixation test. In addition, elevated levels of these antigammaglobulins were frequently associated with a more severe stage of the disease and radiologic evidence of joint destruction. These patients tended to have an unfavorable course and a poor prognosis. Also of interest was the observation that serum complement levels were lower in the sera of patients with elevated antigammaglobulin levels than in those with normal levels. Patients with elevated levels of only IgG and IgA antigammaglobulins had evidence of active disease which generally remitted when levels diminished.

"Cold reactive" rheumatoid factors, belonging to the 19S IgM class of antibodies, were described in the sera of patients with infectious mononucleosis and reticulum cell sarcoma by Capra *et al* (27). These factors, however, were not detected in patients with RA and negative agglutination tests. In an attempt to find a more sensitive serologic technic to analyze patients with JRA, sera from patients with JRA were tested for "cold-reactive" rheumatoid factors. They were indeed found not infre-

quently in patients with JRA and only occasionally in other patients. Those JRA patients with "cold reactive" antigammaglobulins tended to have more severe disease and an unfavorable course.

The complement system has been studied in a number of rheumatic syndromes. Serum whole hemolytic complement levels are elevated in patients with acute rheumatic fever (33), low in many patients with systemic lupus erythematosus (20, 34), and range from low to high in patients with adult rheumatoid arthritis (8, 35). Considerable attention has focused on involvement of complement in the synovial space of patients with RA. Whole hemolytic complement (5, 7, 24), as well as levels of complement components C1, C4, C3 (36), and C9 (37) have been noted to be low in the synovial fluid of patients with RA, especially those with rheumatoid factors (6, 7, 24). This fluid phase depletion of complement is associated with deposition of complement components in conjunction with immunoglobulins in the synovial membrane (14) and in phagosomes of synovial fluid leukocytes (38). These studies, plus those of Winchester *et al*, finding IgG-anti-IgG complexes in rheumatoid synovial fluids (39) support the hypothesis that in adult RA, immune complexes are formed in synovial fluid, activate the complement system, and are subsequently phagocytosed by synovial fluid leukocytes. Activation of the complement system was also associated with severe rheumatoid disease as assessed pathologically and radiologically (40), and was potentiated by the presence of IgM antigammaglobulins (41, 42).

In the present study, high levels of serum complement were noted in JRA patients with active disease. These high levels were seen particularly in patients with RF(−) JRA who also had elevated levels of serum IgG and IgA antigammaglobulins. When these patients with active disease later went into clinical remission, their levels of serum IgG and IgA antigammaglobulins and serum complement fell into the normal range. These results are similar to those noted in adult RA where RF(−)RA, when active, was generally associated with high complement levels (8). The clinical course of both of these high serum complement forms of arthritis is similar in that they appear to be acute, pauciarticular and self-limited.

In addition, in the present study, synovial fluid antigammaglobulins of the IgG and IgA classes were detected in both RF(−) and RF(+) JRA patients. IgM antigammaglobulins were found only in the synovial fluids from patients with RF(+) JRA. Synovial fluid antigammaglobulin levels were greater than those reported for patients with osteoarthritis and comparable to those in adult RA (24). A significant depression of whole hemolytic complement (CH_{50}) as well as complement components C4 and C3 in synovial fluids were noted in patients with RF(+) JRA as compared to patients with RF(−) JRA. In this small number of synovial fluid specimens a trend toward an inverse correlation of synovial fluid IgG antigammaglobulins and synovial fluid CH_{50} levels was observed. In these respects, analysis of synovial fluids from patients with severe JRA reveals changes quite similar to those of adult RA, and suggests that similar mechanisms might be at fault.

Therefore, it seems reasonable to divide patients with JRA into two groups. The first is a small number of JRA patients who have clinical and radiologic findings of severe disease and resemble adult RF(+) RA patients (10–13). As we have shown, these RF(+) JRA patients have elevated levels not only of IgM but also of IgG and IgA antigammaglobulins and reduced

levels of serum and synovial fluid complement. As in adult RF(+) RA, RF(+) JRA patients' exaggerated immune response would seem to be due to the activation of the complement system by these antigammaglobulins and is closely associated with the systemic and articular manifestations of the disease. In the majority of patients with JRA, however, the disease process tends to be self-limited with mild systemic and articular involvement (10–13). As we have shown, similar to the disease pattern, their immune response seems to be also limited and is characterized by increased levels of only IgG and IgA antigammaglobulins and a high serum complement level when they have active disease. These findings suggest that IgG antigammaglobulins may activate the complement system in a different qualitative or quantitative manner than do IgM antigammaglobulins, and that the presence of the former—and "absence" of the latter is associated with a self-limiting and favorable clinical course and a better prognosis in many patients with JRA.

Although one can also consider that there is only one entity, JRA, and that what we have measured represents a continuous spectrum of immune responses to variable inflammatory lesions, we do not know what determines individual qualitative or quantitative immune responses in each patient. It is interesting to note that patients with JRA who lack RF, just as adults with RA, rarely become RF(+) (13). This would argue against a variable spectrum of responses to common inflammatory stimuli, but rather suggest that there might indeed be two forms of JRA, with differing clinical and immunologic manifestations, and perhaps even etiologies.

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