Detection of Immune Complexes in Acute Rheumatic Fever and Their Relationship to HLA-B5

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ABSTRACT Employing five radioimmunoassays for immune complexes, the sera of 45 acute and 27 postacute follow-up sera from patients with acute rheumatic fever were examined. All patients experienced acute polyarthritis. Complexes were detected in 89% of acute-phase sera by one assay, 51% by two, 29% by three, and 7% by four. Immune complex values decreased significantly at followup, although some abnormalities persisted. There was no correlation between extra-articular manifestations and the occurrence of circulating immune complexes. Those positive for HLA-B5 demonstrated a significantly more pronounced immune response as measured by circulating immune complexes. The data indicate that circulating immune complexes occur frequently in adults with acute rheumatic fever. The relative frequency of immune complexes detected by multiple techniques in B5-positive, compared with B5-negative, patients suggests a genetic basis for the development of immune complexes in these patients.

INTRODUCTION

An immunologic response to the group A streptococcus has been strongly implicated in the etiology of acute rheumatic fever (ARF),[1] although the precise pathogenic mechanisms have not been clearly defined (1, 2). In some ways ARF resembles an immune complex-mediated disorder. Signs and symptoms common to both include polyarthritis, fever, and renal abnormalities (3). An immune complex-mediated disorder requires an active humoral response, and, in ARF, antibodies to streptococcal antigens are generally present (4–8). Although most patients have normal or elevated serum complement levels, depressed values have been described in some ARF patients (3, 9) and evidence suggestive of localized complement activation within ARF synovial fluids has been published (10). Therefore, we examined acute and convalescent sera of patients with ARF for immune complexes. Because patients with ARF develop antibodies to multiple cellular and extracellular streptococcal antigens (2, 4–8), a potentially large number of immune complex systems could develop. Therefore, a variety of radioimmunoassays, capable of detecting both complement fixing and noncomplement fixing immune complexes, were used since differing patterns of reactivity have been observed for different types of immune complexes in various disorders (11–13).

A genetic basis for the immune response to many antigens has been defined in experimental animals (14). Greenberg et al. (15) have observed that lymphocytes from HLA-B5-positive individuals were more responsive in vitro to certain streptococcal antigens than were B5-negative lymphocytes. The association of certain HLA antigens with the occurrence of ARF has been examined by several groups (16–21). The results have been somewhat contradictory, and no consistent HLA associations of the A or B loci have been observed. Nevertheless an increased prevalence of HLA-B5 in patients with ARF was noted in several of these reports (16–18, 20). Because ARF appears to result from an immunologic response to the group A streptococcus (1), the association of HLA-B5 with the development of immune complexes in patients with ARF was examined.

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[1] *Abbreviations used in this paper*: ARF, acute rheumatic fever; C1q-BA, C1q-binding assay; C1q-INH, C1q inhibition; HAG, heat-aggregated IgG; mRF, monoclonal rheumatoid factor; mRF-INH, mRF inhibition; PBS, phosphate-buffered saline; PBS-BSA, PBS plus 1% bovine serum albumin; SP, solid phase.

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METHODS

Isolation of C1q

C1q was isolated from plasma by affinity chromatography and gel filtration as described by Kolb et al. (22). Briefly, fresh serum was rendered 10 mM with EDTA and applied to a Sepharose 4B-(Pharmacia Fine Chemicals, Inc., Piscataway, N. J.)-human IgG immunoadsorbent column. After washing extensively with phosphate-buffered saline (PBS) (5 mM sodium phosphate, 0.15 M sodium chloride, 10 mM EDTA, pH 7.4), the C1q was eluted with a linear salt gradient (0.15–1.5 M sodium chloride) in PBS. The C1q was precipitated with ammonium sulfate, resuspended in PBS that contained 0.5 M sodium chloride and eluted over a Bio-gel A-5M (Bio-Rad Laboratories, Richmond, Calif.) column (2.6 × 85 cm) equilibrated in the same buffer. The C1q was pure by radial immunodiffusion and immunoelectrophoresis employing monospecific antisera and an anti-whole human serum.

Isolation of monoclonal rheumatoid factor

A monoclonal rheumatoid factor (mRF) was isolated from serum (kindly provided by Dr. F. C. McDuffie, Rochester, Minn.) by gel filtration over a Sephadex G-200 column (2.6 × 93 cm) eluted with 0.05 M sodium acetate, 0.15 M sodium chloride, pH 3.5. The fractions containing the mRF were pooled, neutralized, and concentrated. This preparation precipitated heat-aggregated but not monomeric immunoglobulin (Ig)G. The mRF was pure when examined by radial immunodiffusion and immunoelectrophoresis.

Antigens and antiserum

Normal pooled human IgG was isolated from Cohn fraction II (Mann Research Laboratories, Inc., New York) by DEAE ion-exchange chromatography and molecular-sieve chromatography. Antibodies to the Fc portion of IgG were prepared by immunization of rabbits with human IgG. The gammaglobulin fraction, obtained by ammonium sulfate precipitation, was pepsin digested (23), dialyzed against a neutral buffer, and eluted over an immunoadsorbent column prepared with isolated human Fc (24). The bound antibodies were eluted with 0.01 N hydrochloric acid, 0.15 M sodium chloride, and neutralized immediately. The antibodies were further purified by elution over IgM, IgA, and Fab(IgG) immunoadsorbent columns. The radiolabeled antibodies were specific for Fc(IgG) by radioimmunoassay.

Isolated antibodies and C1q were radiolabeled by the iodine monochloride method (25) with an ICl:protein ratio of 1:8. Specific activity ranged from 0.10 to 0.25 μCi/μg.

Heat-aggregated IgG (HAG) was obtained by heating Cohn fraction II (10 mg/ml in saline) to 63°C for 20 min. Large aggregates were removed by centrifugation at 1,500 *g* for 15 min.

Radioimmunoassays

C1q-binding assay. The C1q-binding assay (C1q-BA) was performed as described by Zubler et al. (26). As reported by these authors, 2.5% polyethylene glycol was the most useful concentration to discriminate patient sera from control sera. The results were reported as the percentage of the total TCA-precipitable ^{125}I-C1q precipitated by immune complexes in the presence of 2.5% polyethylene glycol.

C1q-inhibition and mRF-inhibition radioimmunoassays. The C1q-inhibition (C1q-INH) and the mRF-inhibition (mRF-INH) assays were performed as described by Gabriel and Agnello (27). The assays quantitate the ability of immune complexes to inhibit the binding of radiolabeled C1q or mRF to normal human IgG coupled to a *p*-azobenzamidoethyl Sepharose-4B immunoadsorbent. Our assays employed a binding buffer with a conductance of 10 mmho/cm instead of 7.0. The results were reported as the percentage of ^{125}I-C1q or ^{125}I-mRF inhibited from binding the IgG-Sepharose by patient or control sera.

C1q-solid phase and mRF-solid phase assays. The solid phase (SP) assays were modified after that described by Hay et al. (28). 0.5 ml of isolated C1q (5 μg/ml) or mRF (1 μg/ml) was incubated in polystyrene tubes (12 × 75 mm, Falcon 2054, Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) overnight at 4°C. After washing three times with 1.0 ml of PBS (5 mM sodium phosphate, 0.15 M sodium chloride, pH 8.0), 0.5 ml of PBS plus 1% bovine serum albumin (PBS-BSA) was incubated in the tubes for 3 h at 4°C. After three additional washes with PBS-BSA, 0.025 ml of test sample, which had been previously incubated in 0.2 M EDTA (pH 7.4) (1:2, v/v) at 37°C for 30 min, was added, and the final volume was adjusted to 0.5 ml with PBS-BSA. The tubes were then incubated at 4°C for 1 h. After washing, 0.5 ml of specific ^{125}I-F(ab')$_2$ rabbit anti-human Fc (1 μg/ml) was added and incubated for 1 h at 4°C. After aspiration and washing, the tubes were counted in an automatic well-type gamma counter. The results were expressed as nanograms anti-Fc bound per tube. Coated tubes to which only buffer was added served as the background controls. All radioimmunoassays were performed in duplicate.

To insure that the radioimmunoassays were capable of detecting immune complexes, the assays were tested using HAG, monomeric IgG, and preformed soluble immune complexes of human IgG, rabbit anti-human IgG, or BSA rabbit anti-BSA. HAG and preformed complexes were detected as immune complexes by each radioimmunoassay. The detection of HAG in normal serum as complexes by the C1q-SP and the mRF-SP is presented in Fig. 1. The C1q-SP was capable of

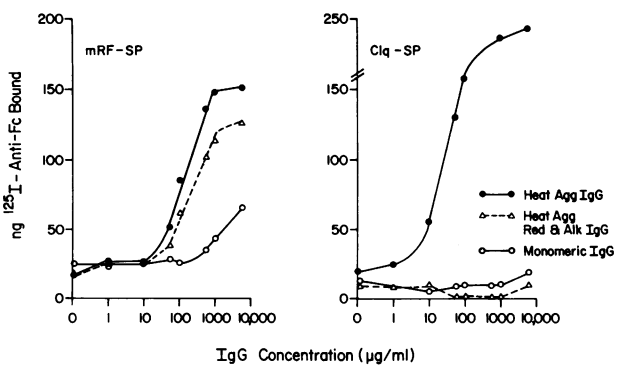

FIGURE 1 Detection of complement fixing and noncomplement fixing immune complexes by the mRF-SP and the C1q-SP. Increasing concentrations of the various IgG preparations were added to normal human serum. Samples were diluted as described in Methods and incubated for 1 h in tubes coated with mRF or C1q. After aspiration and washing, ^{125}I-anti-Fc was added. After a 1-h incubation, samples were aspirated and the tubes washed and counted. Agg, aggregated; Red, reduced; Alk, alkylated.

detecting as little as 0.08 μg (10 μg/ml) of HAG in normal serum, whereas the mRF-SP detected as little as 0.42 μg (50 μg/ml). The assays were also examined for their ability to detect noncomplement fixing immune complexes. When the IgG was reduced and alkylated (29) to prevent complement fixation and then heat aggregated the mRF-SP was still capable of detecting the HAG as immune complexes whereas the C1q-SP was not. Monomeric IgG did not interfere with the C1q-SP. The influence of monomeric IgG on the mRF-SP occurred only at high concentrations, and HAG was detected ≈100-fold more sensitively. This degree of discrimination is similar to that previously described employing mRF to detect immune complexes (27).

Sucrose density-gradient ultracentrifugation

Sera (50 μl) were applied to linear sucrose density gradients (5–20%) prepared in 0.2 M sodium borate, 0.15 M sodium chloride, 0.13 M EDTA, pH 8.0. The gradients were 12 ml total vol and were centrifuged in a Beckman ultracentrifuge (model L5-50, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 35,000 rpm in an SW 41 Ti swinging bucket rotor for 16 h at 4°C. Fractions (0.35 ml) were collected from the bottoms of the gradients. Both 6.6S IgG and 19S IgM served as reference markers in separate gradients during each run. Total protein was determined by the technique of Lowry et al. (30). Radioimmunoassays to detect immune complexes were performed on the gradient fractions following standardization of the protein concentration.

Patients

Sera were obtained from blood clotted at room temperature and frozen at −20°C until use. Samples were obtained from 45 patients with ARF, all of whom fulfilled the modified Jones criteria (31). All patients presented with acute polyarthritis. 9 of the 45 patients had evidence of active carditis at the time of the acute episode. Only six of the patients were <18 yr of age. All were patients at the Bexar County Hospital or Audie Murphy Veterans' Administration Hospital, San Antonio, Tex. All sera were negative for rheumatoid factor by the sensitized sheep-cell agglutination test. All but two sera had an elevated anti-streptolysin 0 test, and the erythrocyte sedimentation rate of all patients was increased, the majority being >100 mm/h (Westegren). Sera of 27 of these individuals were obtained at followup, 2 wk to 7 yr after the acute episode. Only three postacute samples were obtained <1 yr after the acute event. The sera of 16 patients with seropositive rheumatoid arthritis, 18 with systemic lupus erythematosus, and 19 normal individuals served as positive and negative controls.

Miscellaneous

The HLA antigen analysis of 35 ARF patients was performed by the microcytotoxicity assay and has already been reported by Murray et al. (20). There was no significant difference between the patients and the controls in the frequency of any antigen studied. Specifically, HLA-B5 was present in 22% of the 35 patients available for our study compared with 24% of the patients and 16% of the controls in the original group described by Murray et al. (20). IgG concentration was determined by radial immunodiffusion. Student's *t* test and the Mann-Whitney U test were employed to compare ARF and normal means. Fisher's exact test was used to evaluate contingency tables.

RESULTS

Assays with control sera. To insure that the assays were functioning with patient sera as described by others, rheumatoid arthritis and systemic lupus erythematosus sera were employed as positive controls (Table I). The assays detected circulating immune complexes in these sera in patterns similar to those previously described (26–28). For example, the mRF-INH detected immune complexes more sensitively in the rheumatoid sera, whereas the C1q-INH and C1q-SP were more sensitive with the lupus sera. Additionally the relative differences between the normal control values and the patient values were similar to those previously described (26–28).

Assays with ARF sera. The sera of 45 patients with ARF were examined by all five radioimmunoassays. The results, demonstrated in Fig. 2, were obtained by dividing each patient value by the normal control mean for that assay. An immune complex index of 1.0 represents the normal mean for that assay, and the boxes define two standard deviations above and below this

TABLE I

Comparison of Five Radioimmunoassays to Detect Circulating Immune Complexes in Normal, Rheumatoid Arthritis, and Systemic Lupus Erythematosus Sera

	C1q-BA	C1q-SP	mRF-INH	C1q-INH	mRF-SP
	% binding	*ng anti-Fc*	*% INH*	*% INH*	*ng anti-Fc*
Normal (19)*	1.75±0.26‡	12.9±6.03	6.30±4.39	7.90±5.55	22.6±3.83
Rheumatoid arthritis (16)	22.1±26.9 (81%)§	49.9±24.1 (81%)	18.6±10.9 (69%)	20.3±15.3 (50%)	48.8±21.7 (75%)
Systemic lupus erythematosus (18)	11.5±14.0 (83%)	87.0±56.0 (89%)	8.69±8.49 (22%)	36.1±17.6 (83%)	41.7±18.4 (67%)

* Number of sera examined.

‡ Means±1 SD.

§ Percentage of values >2 SD above the normal mean.

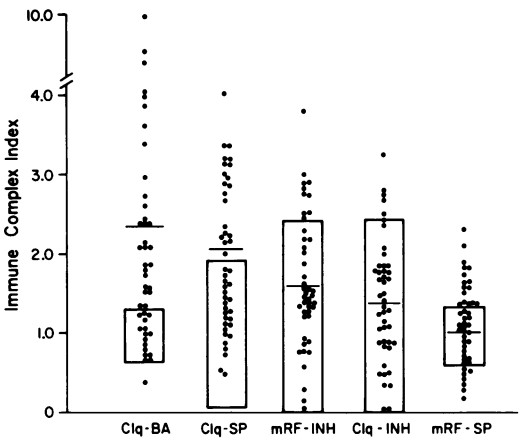

FIGURE 2 Distribution of immune complex values by five radioimmunoassays in patients with ARF. Each point represents the mean of an individual patient. The immune complex index was obtained by dividing each patient mean by the control mean for that assay. A value of 1.0 represents the normal mean for that assay. The horizontal lines represent the ARF means. The enclosed boxes represent the normal mean±2 SD for each assay.

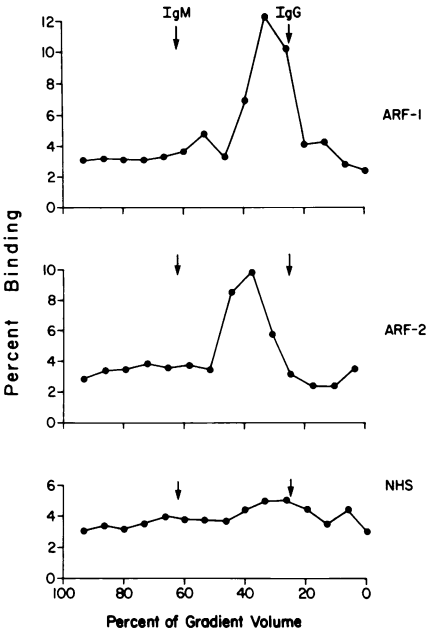

FIGURE 3 Characterization of the molecular weight of immune complexes detected by the Clq-BA after preparative ultracentrifugation. The sera of two patients with ARF and of one normal control (NHS) are demonstrated. The Clq-BA on the untreated sera of the patients was 3.7% (ARF-1) and 11.2% (ARF-2) while the control was 1.95%. Linear sucrose density gradients were prepared with 5–20% sucrose in borate buffer, pH 8.0, and centrifuged for 16 h at 35,000 rpm in an SW 41 Ti swinging bucket rotor. Direction of centrifugation is from right to left. The positions of the 19S and 6.6S markers run simultaneously in a separate gradient are indicated at the top of the figure.

mean. The mean of the ARF sera was significantly different from the normal controls by the Clq-BA ($P < 0.01$), the Clq-SP ($P < 0.001$), and the mRF-INH ($P < 0.05$). 64% of ARF sera were abnormally elevated by the Clq-BA, 44% by Clq-SP, 22% by the mRF-INH, 11% by Clq-INH, and 36% by mRF-SP. 89% of patients were abnormal by one assay, 51% by two, 29% by three, and 7% by four.

The size characteristics of the immune complexes were examined after preparative ultracentrifugation employing sera of six patients with ARF. The distribution of immune complex-like materials for ARF and normal sera by the Clq-BA, the Clq-SP, and the mRF-SP assays are demonstrated in Figs. 3–5. Immune complexes in ARF sera sedimented predominantly between the 6.6 and 19S markers. By each assay the greatest quantity of activity was present in this intermediate range. Complexes sedimenting faster than 19S were not detected by the Clq-BA and only in two sera by the Clq-SP. The mRF-SP detected some activity in the >19S region of all six patients. The two sera with the most >19S material are presented in Fig. 5. Small peaks of activity in the 6.6S region were present with normal sera. This was most apparent with the SP assays and was most likely a result of monomeric IgG. To further document the presence of immune complexes, an ARF serum possessing intermediate-sized immune complexes was subjected to sucrose density ultracentrifugation under dissociating conditions. After neutralization, the peak of immune complex activity was no longer detected (Fig. 6).

All patients with ARF presented with a migratory polyarthritis, fever, and an elevated erythrocyte sedimentation rate. Most also had an elevated anti-streptolysin 0 titer. There was no association of extra-articular manifestations such as carditis or minor renal or liver functional abnormalities with the presence circulating immune complexes.

The relationship between the values obtained for patients with ARF by the five radioimmunoassays was examined. The Clq-SP correlated with Clq-BA ($P < 0.05$), mRF-INH ($P < 0.005$), and the mRF-SP ($P < 0.001$), whereas the mRF-INH also correlated with the mRF-SP ($P < 0.001$). No relationship was observed between any immune complex assay and the anti-streptolysin 0 titer or the erythrocyte sedimentation rate. The only association between immune complexes and IgG concentration was for the Clq-BA ($r = 0.4555$, $P < 0.01$). The Clq-BA assay was not effected by the addition of monomeric IgG to normal human sera. Although the assays employing mRF were influenced by the similar addition of monomeric IgG (Fig. 1), no correlations existed between the mRF-SP or mRF-INH assays and the IgG concentrations of our patients.

Postacute sera. Follow-up sera were available on 27 of the ARF patients. The mean values for the paired

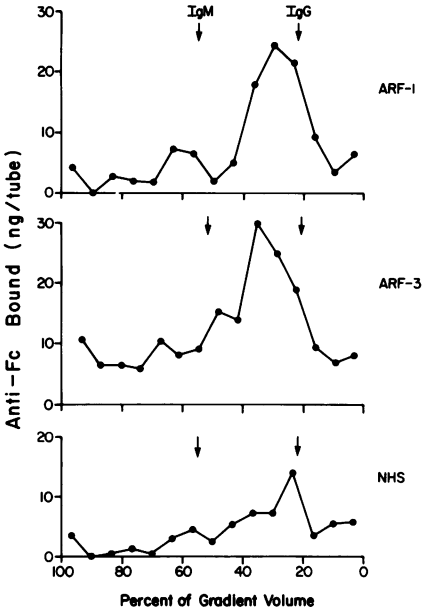

FIGURE 4 Characterization of the molecular weight of immune complexes detected by the C1q-SP after preparative ultracentrifugation. The C1q-SP on the serum of ARF-1 was 43.8 ng anti-Fc and of ARF-3 was 57.2 while the control (NHS) was 13.2. Gradients were prepared as described in Fig. 3.

acute sera are presented in Table II. The mean values for the C1q-BA and C1q-SP were significantly diminished from the acute phase, whereas the other three assays did not change significantly. The acute and follow-up values for the individual patients who are initially abnormal by the C1q-BA, C1q-SP, and the mRF-

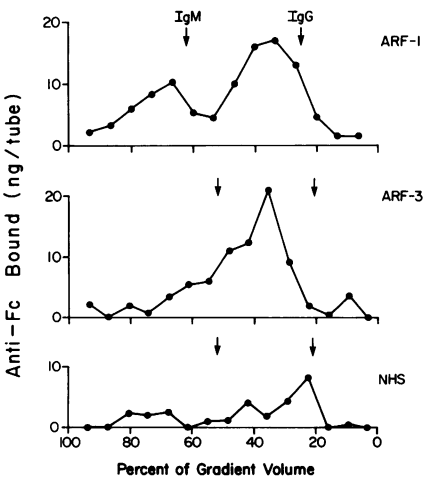

FIGURE 5 Characterization of the molecular weight of immune complexes detected by the mRF-SP after preparative ultracentrifugation. The mRF-SP on the patient sera were 52.9 and 39.2 ng anti-Fc while the control serum (NHS) was 16.5. Gradients were prepared as described in Fig. 3.

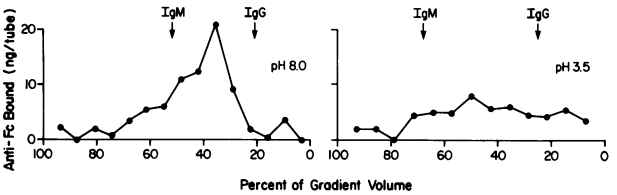

FIGURE 6 The effect of ultracentrifugation under dissociating conditions on the detection of immune complexes by the mRF-SP. The gradients were prepared with 5–20% sucrose. The pH 8.0 gradient was prepared with borate buffer and the pH 3.5 gradient with 0.05 M sodium phosphate, 0.15 M sodium chloride. After centrifugation, the pH 3.5 gradient fractions were adjusted to pH 7.5 with 1 N sodium hydroxide. The remainder of the assay was performed as already described.

INH are presented in Fig. 7. All but one determination had decreased in the postacute phase. Of interest, the follow-up values for the C1q-SP and the mRF-INH assays were still significantly different from the normals ($P < 0.05$), although only a few individual samples were outside the 95% confidence limits of the controls.

Relation of HLA-B5 to the presence of immune complexes. We examined the relation of HLA-B5 to the presence of immune complexes. The HLA antigen analysis of the A and B loci for 35 of our patients has been included in the report of Murray et al. (20). 10 of the 35 patients were HLA-B5 positive. The mean values for four of the five radioimmunoassays were higher in the B5-positive group. None of these differences was significant, although that for the C1q-SP approached significance ($P < 0.065$). The frequency of the detection of immune complexes by multiple assays was compared for the B5-positive and -negative patients (Fig. 8). The frequency of complexes by two and three assays is presented not only for the HLA-typed patients but also for the normal controls, and all acute and postacute or convalescent ARF samples. Immune complexes were detected in 51% of all ARF patients by two techniques and in 29% by three. One follow-up sample was positive by three methods. This specimen was obtained only 4 mo after the acute episode. This was a B5-negative individual who also had three positive assays in the acute phase. There was no instance of immune complex detection by multiple assays in the controls. Complexes by two assays were present in 70% of the B5-positive and 36% of the B5-negative patients, whereas 70% of B5-positive and only 12% of B5-negative patients had immune complexes by three techniques. This difference was significant, $P = 0.0016$. The frequency of immune complex detection in relation to other HLA antigens was also compared. Except for two individuals positive for HLA-A11, each of whom had three assays positive, no other significant correlations were found. The association between the frequency of immune complex abnormalities and HLA-

TABLE II
Comparison of 27 Paired Acute and Convalescent ARF Sera

	Clq-BA	Clq-SP	mRF-INH	Clq-INH	mRF-SP
	% binding	*ng anti-Fc*	*% INH*	*% INH*	*ng anti-Fc*
Acute	4.94±3.82*	25.1±12.7	10.2±3.87	8.95±5.55	25.2±9.48
Convalescent	1.79±0.71 ($P < 0.001$)‡	18.0±6.34 ($P < 0.005$)	10.0±3.28 (NS)	9.06±4.28 (NS)	23.0±9.05 (NS)

* Mean±1 SD.
‡ Significance of difference from acute phase values.

B5, when corrected for the total number of antigens available (15), remained significant ($P < 0.05$).

DISCUSSION

The data clearly demonstrate that immune complex-like materials were present in the sera of the majority of our patients with ARF. The results of preparative ultracentrifugation support this conclusion. The diminution of abnormal values at followup indicates that the majority of the complexes were associated with the acute event. Our findings are in agreement with the report of van de Rijn et al. (32), which described immune complexes in patients with ARF and poststreptococcal glomerulonephritis.

If the complexes detected in patients with ARF are responsible for certain manifestations, such as the polyarthritis, they might be expected to be large-sized complexes (≥19S), analogous to those responsible for experimental immune complex disease (33, 34). The circulating complexes detected in our patients were primarily of low molecular weight (≤19S), although larger complexes were detected. Van de Rijn et al. (32) detected both large and small molecular weight complexes in two patients with ARF. They observed a similar size distribution of complexes in patients with poststreptococcal glomerulonephritis. Patient selection may have accounted for the differences between the two studies. All patients in their study were under 15 yr of age, whereas most of our patients were adults. Also, all their patients were from the island of Trinidad, whereas the background of our patients was more diverse. Variability of immune response to the streptococcal antigens or of the rate of immune complex clearance may have accounted for the differences in the relative quantities of large-sized complexes between the two studies. The possibility that large molecular weight immune complexes had already deposited at the time of our observations cannot be excluded. In experimental animals, IgG-containing immune complexes sedimenting between 14 and 22S were cleared rapidly by the reticuloendothelial system whereas smaller complexes persisted much longer (35). Therefore, large-sized complexes should not be detected for long periods of time after further pro-

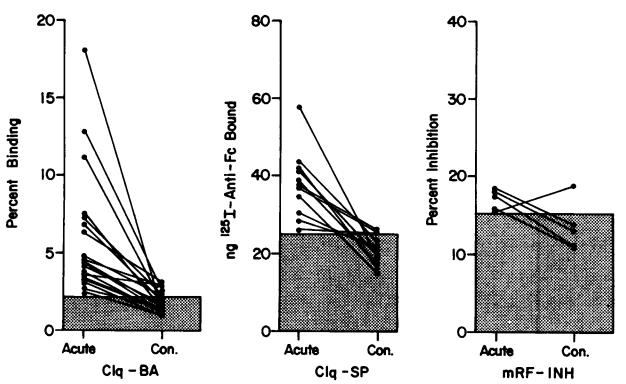

FIGURE 7 Comparison of acute and postacute followup ARF sera that were initially abnormal by Clq-BA, the Clq-SP, and the mRF-INH. The results for each assay are reported as described in Methods. The stippled areas encompass the upper limits of normal for each assay. Con. refers to the convalescent or postacute period.

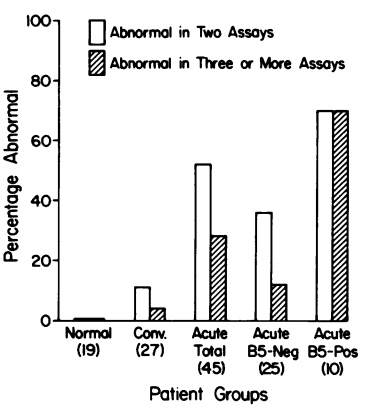

FIGURE 8 Frequency of multiple immune complex abnormalities in patients with ARF and their relationship to HLA-B5. The patient groups analyzed and their numbers are represented along the abscissa. The open bars represent the percentage of patients in each group with two positive or abnormal radioimmunoassays and the striped bars the percent with three. Con. refers to the postacute period. B5-Neg, B5-negative; B5-Pos, B5-positive.

duction of complexes has ceased. This may account for the relative paucity of larger-sized immune complexes in our study.

If immune complexes are of direct pathogenic significance in ARF they might be expected to activate complement (33). Some patients with ARF have depressed serum complement (3, 9), although the majority have normal or elevated levels (9, 10, 36). However, because several of the complement components behave as acute-phase reactants, measurement during ARF might not detect activation even if present. This situation has been observed in immune complex-mediated disorders such as rheumatoid arthritis. In this disorder, circulating immune complexes have frequently been detected (27, 37) even though complement has generally been normal or elevated (36, 38, 39). The presence of activated complement products (40) and the accelerated turnover of certain complement components (41–43) demonstrated that complement consumption occurs in patients with rheumatoid arthritis. Similar studies in ARF will more precisely define the potential role of circulating immune complexes in the pathogenesis of certain manifestations.

It is possible that a localized immune complex-mediated disorder might occur within the joints in ARF, similar to rheumatoid arthritis. The synovial fluids of patients with rheumatoid arthritis contain immune complexes as well as decreased complement and activated complement components (37, 44). Synovial fluids from five of our ARF patients were examined for immune complexes and these values were compared with 14 synovial fluids from patients with degenerative joint disease, gout, and pseudogout. No significant differences were detected and only one assay from one patient with ARF was positive. Complexes were detected in the sera of three of these patients by two assays and by one assay in the other two. Firm conclusions cannot be drawn from these observations because of the limited number of samples available for analysis. Svartman et al. (10) quantitated complement and other proteins in the sera and synovial fluids of 25 patients with ARF. Their data were interpreted as indicating a localized consumption of the early components of complement within the synovial fluids, possibly by immune complexes. Further studies to detect immune complexes and complement activation products within ARF synovial fluids will be necessary to determine if a localized immune complex-mediated synovitis occurs in this disorder.

The pathogenesis of ARF is unclear. The humoral (4–8, 45) and cellular (46) responses to streptococcal antigens observed in ARF patients suggest a crucial role for this agent (1). However, the precise pathogenic mechanisms responsible for the various presentations of ARF may be different. Humoral or cell-mediated immunity directed against the streptococcus that is cross-reactive with heart (45–49) and other tissue antigens (2) or immune complexes or a combination of these may be responsible for the various presentations of the disease. Even if immune complexes are not directly responsible for certain manifestations of ARF, such as the polyarthritis, they may be important in modulating the immune response (12, 50–52) to streptococcal antigens in ARF and, therefore, involved in the pathogenesis.

The results observed with the postacute sera were of particular interest. Most values diminished at followup, although significant differences from the normal controls persisted. These observations were in agreement with those of van de Rijn et al. (32). The C1q-SP values decreased significantly although the mean remained significantly different from the controls and 5 of 27 samples were still abnormal. The mRF-INH postacute means were also significantly different from the controls even though only one sample was abnormal at followup. Only one postacute sample had immune complexes detected by three assays whereas they were detected in three by two techniques. The sample with three positive radioimmunoassays was obtained only 4 mo after the acute event. Although no synovitis was apparent on examination, the patient complained of polyarthralgia and myalgia and had significant valvular disease requiring valve replacement 7 mo later. Because multiple antibody systems can persist for 6 mo to 2 yr or longer (4, 8, 47), especially in patients with valvular disease (8), the persistence of immune complexes in this individual was not entirely surprising. The reason for the difference between the ARF postacute and the control sera is unknown. Possibly, these individuals are more likely to respond to normal microflora and other antigens even in health. A persistent immune responsiveness to streptococcal antigens, both humoral (4, 8, 47) and cell mediated (46), has been observed in ARF patients, even 5 yr after the acute event. The persistence of differences between the controls and the postacute ARF sera in our study, although not specific, is consistent with the concept of a prolonged or exaggerated immune responsiveness to streptococcal or other antigens in patients with ARF (1).

The previous observation of an increased in vitro response to streptococcal antigens by lymphocytes from HLA-B5-positive individuals (21) prompted us to examine the relation of HLA-B5 to the presence of immune complexes in our patients. Because patients with ARF have had a recent streptococcal infection, it seemed possible that the B5-positive ARF patients might exhibit a more marked immune response as measured by immune complexes. Not only were the mean immune complex values higher in four of the five radioimmunoassays for the B5-positive patients, but, also, complexes were detected by multiple assays significantly more often. This association was not detected

for any of the other HLA-A or -B antigens determined. Several explanations of these findings are possible. One explanation relates to heterogeneity of the immune response. Multiple antibody systems with differing specificities, such as antibodies against steptolysin 0, streptococcal hyaluronidase, and DNAase, or against group-specific carbohydrate have been demonstrated in patients with ARF (2, 4–8, 47). The increased frequency of immune complexes detected by multiple radioimmunoassays in the B5-positive patients may suggest a more heterogeneous immune response with the development of multiple antigen-antibody systems in these individuals.

The methods we employed have clearly demonstrated differing specificities regarding the types of immune complexes detected (12, 13, 27, 53). For example, the C1q-BA will more sensitively detect certain low molecular weight complexes (13). Also, the C1q-BA detects both IgM- and IgG-containing immune complexes, whereas the other assays detect only those containing IgG. The C1q assays detect only complement fixing complexes; mRF assays can detect both complement and noncomplement fixing complexes. Therefore, the assays may be detecting, at least in part, different immune complex systems. Because multiple assays were positive in our B5-patients, this suggests a greater response to streptococcal antigens in these individuals.

Many substances other than immune complexes may affect the radioimmunoassays. For instance, heparin and DNA may influence the assays employing C1q, whereas rheumatoid factor may interfere particularly with those using mRF. Therefore, because the techniques employed were antigen nonspecific and because substances other than immune complexes may influence them, detection of complexes in any given sample by multiple methods is more likely to truly reflect the presence of immune complexes than detection by only one assay.

It is unlikely that the radioimmunoassays were selecting out differences related specifically to HLA-B5, because they were not antigen specific and no cellular materials were employed. Additionally, not all B5-positive individuals had detectable immune complexes acutely, and at postacute followup there was no difference between the B5-positive and -negative groups.

The HLA-B5-positive ARF patients developed a more intense response in the face of an antecedent streptococcal infection as measured by immune complexes than HLA-B5-negative patients. The genetic marker HLA-B5 was not associated with the development of ARF. Neither the assays for immune complexes nor the HLA analyses selected out differences in clinical presentation. All our patients presented with acute polyarthritis, and none had other manifestations without arthritis. Of the nine patients with active carditis, four patients had three positive assays while three had one or two. Eight of the carditis patients were HLA typed and four were B5-positive. Of these four, three had immune complexes detected by three assays, while the other was positive by only one. Other extra-articular manifestations such as transient renal (six patients) and hepatic (two patients) functional abnormalities were less frequent and not associated with differences in immune complexes.

In summary, circulating immune complexes are present in the majority of patients with ARF. The pathogenic importance of these complexes remains unsettled. Nevertheless, the association of HLA-B5 with a greater frequency of abnormalities supports the concept of a linkage disequilibrium between HLA-B5 and the immune response to streptococcal antigens.

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