IgG anti-IgG antibodies in rheumatoid arthritis and certain other conditions

JOHN L. ABRUZZO AND RALPH HEIMER
From the Departments of Medicine and Biochemistry, Jefferson Medical College, Thomas Jefferson University, Philadelphia, U.S.A.

Rheumatoid factor (Rose, Ragan, Pearce, and Lipman, 1948), known to be a frequent finding in the sera of patients with rheumatoid arthritis, was thought to be an unusual antibody in that it occurred in the 19S and 22S forms (Franklin, Holman, Müller-Eberhard, and Kunkel, 1957) without showing evidence for 7S analogues. The activity of this antibody appeared to be directed against gamma-globulin.

It was not until the early 1960s that 7S antibodies, presumably of the IgG class, with similar activity were also noted. These were first described (Kunkel, Müller-Eberhard, Fudenberg, and Tomasi, 1961) as circulating intermediary 9-17S) complexes composed of 7S gammaglobulin monomers, occurring in a few selected cases of severe rheumatoid arthritis. Additional evidence of their existence was later presented by Chodirker and Tomasi (1963) and Heimer and Levin (1966), but it was not until 1967 that Torrigiani and Roitt (1967), with a combination of immunoadsorption and radial immunodiffusion with specific antisera, reported the wide occurrence of IgG anti-IgG antibodies in rheumatoid sera. There is evidence (Winchester, Agnello, and Kunkel, 1970) to suggest that IgG anti-IgG antibodies may have an important role in the pathogenesis of the chronic synovial inflammation which characterizes rheumatoid arthritis.


Previously we have described a modified procedure of immunoadsorption and a simple tube-dilution latex test for the detection of IgG anti-IgG antibodies (Heimer and Abruzzo, 1972) which, in the conditions employed by us, is specific for the detection of IgG anti-IgG.

In this study we demonstrate the reproducibility of this latex test and report the occurrence of IgG anti-IgG antibodies in rheumatoid arthritis, osteoarthritis, hepatitis, and primary biliary cirrhosis.

Material and methods

PREPARATION OF IMMUNOADSORBENT

1 g. human IgG (Fraction II, Squibb Laboratories) was dissolved in 20 ml. phosphate buffer (pH 7-2, 0-1 M) and heated to 63°C. For 10 min. After cooling, 4 ml. of 2-5 per cent. aqueous glutaraldehyde was added drop by drop with stirring, following the general procedure of Avrameas and Ternynck (1969). The mixture was allowed to stand for 2 hrs at room temperature, and the resultant gel was homogenized in a blender in the presence of ammonium formate buffer (pH 3-0, 0-1 M). The homogenized material was distributed in 40-ml. plastic test tubes, each containing approximately 125 mg. immunoadsorbent. The latter was suspended in phosphate buffer (pH 7-0, 0-1 M) and stored at 4°C, until used. The immunoadsorbent could be used repeatedly, but care had to be exercised in removing all previously adsorbed material by washing with pH 3-0 buffers.

SERUM IMMUNOADSORPTION

Sera were used either when freshly obtained or upon storage in the frozen state. 1 ml. serum was exposed to 0-05 M mercaptoethanol for 1 hr at room temperature and then diluted with phosphate buffered saline to 30 ml. The solution was added to 125 mg. immunoadsorbent and shaken at room temperature for 1 hr. The tubes were then centrifuged and the supernatant discarded. The immunoadsorbent was washed usually four times with 30 ml. phosphate-buffered saline. The criterion for the final wash was an absorbance of less than 0-005 at 280 mμ in a 1 cm. light path. IgG anti-IgG was then eluted in 10 ml. ammonium formate buffer (pH 3-0, 0-1 M). A single such extraction usually accounted for approximately 80 per cent. of all recoverable IgG. In order to avoid further dilution of

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Reprints requests should be addressed to: John L. Abruzzo, M.D., Thomas Jefferson University, 1025 Walnut Street, Philadelphia, Pa. 19107 U.S.A.
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eluate, additional extractions were not carried out. The eluate was brought to pH 8.4 with the addition of concentrated NH₄OH.

**LATEX AGGLUTINATION**

Materials eluted from the immunoabsorbent were neutralized and serially diluted in 0.5 ml. phosphate buffer (pH 8.4, 0.1 M). The latex reagent was prepared with No. 615 Lytron particles (0.2 μ diameter, Monsanto Chemical Co.), suspended in phosphate buffer (pH 8.4, 0.1 M), and adjusted in a Hitachi-Perkin Elmer spectrophotometer to an absorbance of 0.80 at 400 μμ in a 1 cm. light path. After addition of 0.5 ml. reagent to each tube, the mixtures were incubated at 56°C for 30 min. and read after standing overnight at room temperature. The agglutination patterns were stable for at least 3 days.

**POPULATION**

Serum samples were obtained from 38 persons with definite or classical rheumatoid arthritis (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959). Thirty had serum rheumatoid factor titres of 1:80 or greater. Eight were negative for serum rheumatoid factor or had titres of less than 1:80 as measured by the latex test of Singer and Plotz (1956). The patients were selected only on the basis of adequate clinical data and availability of serum.

Serum samples were also obtained from thirty persons with osteoarthritis and from twelve healthy young adults randomly selected from among hospital employees. There were nineteen sera from patients with hepatitis, nine of whom were positive for Australia antigen, and five patients with primary biliary cirrhosis. The diagnosis of hepatitis and primary biliary cirrhosis was based on typical clinical and laboratory features combined with liver biopsy histology consistent with those entities.

The mean age of the rheumatoid arthritis group that was rheumatoid factor positive was 60 years. The mean age of the rheumatoid factor negative subgroup, the osteoarthritis group, and the healthy young adult group was 51, 65, and 25 years respectively. The mean age of the hepatitis group was 34 years and that of the primary biliary cirrhosis group 47 years.

**Results**

In order to determine the reproducibility of our method of testing IgG anti-IgG antibody, eluates recovered after immunoadsorption of a serum of a patient with rheumatoid arthritis were tested on eleven separate occasions by two technicians independently in two separate laboratories. Serial dilution was started with an aliquot equivalent to a 1:10 dilution of the original serum. A high degree of reproducibility was achieved (Table). The mean titre was 4.77 tubes agglutinated with a standard deviation of ±0.47.

Having established the reproducibility of the agglutination test, we then analysed a panel of sera obtained from our clinic population. As the Figure (overleaf) shows, the highest titres of IgG anti-IgG antibody were found within the rheumatoid group. The geometric mean titre in the rheumatoid group was 1:79 compared with 1:27 in the osteoarthritis group. The difference in titres between these two groups is highly significant (P (Student's test) less than 0.001). The geometric mean titre in the healthy young adult group was 1:15. The range of individual titres within each group was such that the rheumatoid and the healthy young adult control groups had few overlapping values. The osteoarthrisis group, on the other hand, contained a wide range of titres that overlapped with the other two groups.

Our results appear to be consistent with those obtained by other methods of purification of IgG anti-IgG followed by quantitation using radial immunodiffusion (Torrigiani and Roitt, 1967; Torrigiani and others, 1970; Panush and others, 1971; Tapanes and others, 1972).

The geometric mean titre of the IgG anti-IgG antibody in the hepatitis group was 1:27 and was evidently independent of the simultaneous presence or absence of Australia antigen. The geometric mean titre as well as the range was similar to that observed in the osteoarthrisis group and significantly greater than that of the healthy young adults whose age range is similar to that of the hepatitis group.

No evidence of significant titres of IgG anti-IgG was found in primary biliary cirrhosis. The highest titre in this group was 1:20, well within the range measured in the healthy young adults. The number of patients with primary biliary cirrhosis, however, is small and does not allow us to draw a definite conclusion.

**Table**

<table>
<thead>
<tr>
<th>Test no.</th>
<th>Number of tubes agglutinated*</th>
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<tr>
<td>1</td>
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<td>2</td>
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<td>11</td>
<td>5.0</td>
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</table>

ΣXi = 52.5  
Mean = 4.77 tubes  
Standard deviation = ±0.47 tubes

*"Tubes agglutinated" represents the number of tubes containing serially diluted eluate showing agglutination. If the last tube was rated "trace agglutination", it was recorded as 0.5, hence the 4.5 recorded in the Table. As the first tube tested represented a dilution of 1:10, four tubes is equivalent to 1:80, 4.5 tubes to 1:120, and five tubes to 1:160.

**Discussion**

IgG anti-IgG antibodies can be found in relatively low concentrations in the serum of many patients with rheumatoid arthritis (Torrigiani and Roitt,
1967; Torrigiani and others, 1970; Panush and others, 1971; Tapanes and others, 1972). These results are confirmed by our study, which employed a modified immunoadsorbent for isolation and latex agglutination for quantitation of IgG. Our test system is based on the observation (Heimer and Abruzzo, 1972) that suspensions of certain preparations of latex particles are agglutinated when exposed to even very small amounts of IgG anti-IgG antibody in the absence of inhibiting substances. The presence of other IgG appears to inhibit the agglutination of the test particles by IgG anti-IgG antibody, hence the isolation of IgG anti-IgG antibody by immunoadsorption is a necessary procedure. Our test appears to have certain advantages over previously published methods for detection of IgG anti-IgG antibody. The immunoadsorbent composed of heated and glutaraldehyde cross-linked IgG is more efficient than cross-linked IgG which has not been heated (Heimer and Abruzzo, 1972). Pre-treatment of the serum with 0·05 M mercaptoethanol and 30-fold dilution of the sample before exposure to the immunoadsorbent renders eluates free of detectable IgM and Clq (Heimer and Abruzzo, 1972). While serum albumin was occasionally observed to be a trace contaminant, even highly concentrated eluates failed to react with three different anti-IgM preparations. Our latex test is sensitive in that serum concentrations of IgG anti-IgG antibodies as low as 5 μg./ml give a titre of 1:10 (Heimer and Abruzzo, 1972). Furthermore, the test results are quite reproducible and as no final concentration step is required as is for radial immunodiffusion, some labour is saved and potential errors usually associated with concentration procedures are avoided. The cost of a latex agglutination test is negligible when compared with that of radial immunodiffusion, and since the test is so very sensitive, one can elect to do radial immunodiffusion and latex agglutination at the same time. Our test has the disadvantage in regard to quantitation that is inherent in all serial dilution procedures, but our results indicate that this disadvantage appears to be of little consequence.

Our finding of IgG anti-IgG antibodies in patients with chronic active viral hepatitis and their relative absence in cases of primary biliary cirrhosis is of interest. It is not surprising that IgG anti-IgG antibodies exist in diseases other than rheumatoid arthritis. IgM anti-IgG antibodies have been substantiated in patients with viral hepatitis (Risemberg, DeGomez, and Rife, 1969) and anti-IgG antibody production is probably the result of stimulation by denatured IgG or IgG present in an immune complex (Milgrom and Witebsky, 1960; Abruzzo and Christian, 1961; Aho and Wager, 1961; Williams and Kunkel, 1963). Hence it is possible that Australia antigen and presumably additional antigens operative in viral hepatitis when complexed with antibody lead to the stimulation of IgG anti-IgG antibodies. If the essentially negative response observed in biliary cirrhosis can be confirmed, it would suggest that there may be no stimulus for anti-IgG production in that disorder active at the time of our testing. It would be of interest in this regard to examine patients with early active disease in order to examine that question more thoroughly.

It is not surprising that a relatively large percentage of patients with osteoarthritis were found to have increased titres of IgG anti-IgG antibody in their serum. Persons with osteoarthritis are generally older and titres of IgG anti-IgG antibody activity may reflect a cumulative, more varied, and prolonged immunological host experience with a multiplicity of antigens. Additional evidence for this hypothesis is the known fact that IgM anti-IgG antibodies (rheu-
matoid factor) occur in older persons (Heimer, Levin, and Rudd, 1963) in significantly greater quantities than in healthy young adults.

Summary

A modified technique of immunoabsorption and a new simple latex test were used for the detection of serum IgG anti-IgG antibodies. As used by us, the latex test is both specific and reproducible. 38 subjects with rheumatoid arthritis, thirty with osteoarthritis, nineteen with hepatitis, and five with primary biliary cirrhosis, and twelve healthy adults were examined for serum IgG anti-IgG antibody. The highest titres were found in the rheumatoid arthritis group; the geometric mean titre being 1:79. The geometric mean titres in the osteoarthritis and hepatitis groups were both 1:27 and in healthy adult controls 1:15.

The possible significance of serum IgG anti-IgG antibodies in these various groups is discussed.

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References


AVRAMEAS, S., AND TERNYNCK, T. (1969) Immunochemistry, 6, 53 (The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoabsorbents)

BIANCO, N. E., PANUSH, R. S., STILLMAN, J. S., AND SCHUR, P. H. (1971) Arthr. and Rheum., 14, 685 (Immunologic studies of juvenile rheumatoid arthritis)


HEIMER, R., AND ABRUZZO, J. L. (1972) Immunochemistry, 9, 921 (A latex test for the detection of human IgG aggregates and IgG anti-IgG antibody)

— AND LEVIN, F. M. (1966) Ibid., 3, 1 (On the distribution of rheumatoid factors among the immunoglobulins)


PANUSH, R. S., BIANCO, N. E., AND SCHUR, P. H. (1971) Arthr. and Rheum., 14, 737 (Serum and synovial fluid IgG, IgA and IgM antigammaglobulins in rheumatoid arthritis)


— AND ROITT, I. M. (1967) Ibid., 26, 334 (Antiliglobulin factors in sera from patients with rheumatoid arthritis and normal subjects)


