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Antigammaglobulins in Normal Individuals and in Patients with Adult and Juvenile Rheumatoid Arthritis and Osteoarthritis [1]

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I. Introduction

Rheumatoid factors or antigammaglobulins have been demonstrated in the sera of the majority of patients with rheumatoid arthritis by the agglutination of sensitized particles coated with γ-globulins [9, 17, 29]. The presence of rheumatoid factor as measured by these agglutination tests correlate with some but not all clinical features of rheumatoid arthritis. These agglutination tests, however, provide only a semiquantitative technique of the amount of antigammaglobulins present. The development of new immunoadsorbent methods [1, 8, 26–28] facilitating the isolation of antibodies and their quantitation prompted us to reexamine the estimation of antigammaglobulins in patients with rheumatoid arthritis and to correlate these quantities with clinical features of the disease. It is the purpose of this paper to review our results from the quantification of IgG, IgA and IgM antigammaglobulins in the serum of normal individuals and in the serum and synovial fluids from patients with osteoarthritis (OA) and in the serum and synovial fluids in patients with rheumatoid arthritis (RA) [3, 16]. Furthermore, both IgG and IgM antigammaglobulins were isolated from these

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groups of individuals and tested as regards to precipitation, latex agglutination and complement fixation [2].

II. Materials and Methods

A. Population

Sera was obtained from 50 normal individuals, 49 patients with OA, 143 patients with RA and 148 with juvenile rheumatoid arthritis (JRA). Synovial fluids were available on 18 patients with OA, 52 with RA and 16 with JRA.

B. Clinical Studies

Hospital records were reviewed and the following noted: patient's age, sex, age at onset, duration of disease, presence of subcutaneous nodules, fever, weight loss and uveitis, lymphadenopathy, hepatomegaly, splenomegaly, pericarditis, pleuritis and joint involvement characterized as monarticular or polyarticular, particularly for the JRA patients. The total number of inflammed, painful or joints with limited range of motion was counted. The presence of peripheral neuropathy or vasculitis of the skin was noted. The stage of the disease and functional class [13, 25] was assessed. Radiological findings of erosion in the joint space, narrowing, degenerative changes, ankylosis, dislocation and subluxation were recorded. The use of various medications was tabulated. The number of criteria considered diagnostic for RA was counted [19].

C. Laboratory Studies

The following laboratory studies were performed: complete blood count, blood sedimentation index, serum protein electrophoresis, LE preparation, antinuclear antibody test, serum and synovial fluid whole hemolytic complement (CH50) levels and complement components [20], synovial fluid analysis for white blood cells, protein and mucin clot formation, protein and glucose levels [18] and phagosomes [4].

D. Immunological Studies

Human IgG was prepared by ammonium sulfate fractionation of Cohn fraction II[3]. The IgG was insolubilized with glutaraldehyde [1] or in later experiments following heat aggregation coupled to cyanogen bromide-activated sepharose 6B [8]. The immunoadsorbent (IA) was then washed thoroughly with 0.01 M phosphate buffered saline, pH 7.2 (PBS) until the washes no longer contained proteins as determined by a negative optical density at 280 nm. Varying amounts of heat inactivated serum or synovial fluid were

3 Kindly given by Lederle Labs, Pearl River, N.Y.

incubated with the IA at 37 °C for 1 h and 18 h at 4 °C. The preparations were then thoroughly washed with cold PBS, either by centrifugation or chromatography, until washes had a zero OD at 280 nm. Supernatants were saved. Antigammaglobulins were eluted with either pH 4.5, 0.1 M sodium acetate or pH 2.8, 0.01 M glycine HCl buffers. Eluates were immediately neutralized with dilute sodium hydroxide, dialyzed versus PBS, filtered and concentrated by negative pressure dialysis. The protein content in the eluates were determined by the Folin method [14]. Eluates were analyzed by immunoelectrophoresis [21] with antisera to individual proteins and to whole human serum. The concentrations of IgG, IgG subclasses, IgA, IgM and human serum albumin (HSA) in serum and/or eluates was determined by radial immunodiffusion [15, 24], utilizing monospecific antisera. As little as 10 μg/ml of IgG, IgA and 20 μg/ml of IgM and 5 μg/ml of HSA could be detected. IgG, IgA and IgM antigammaglobulins were isolated in large amounts from several hundred milliliters of sera from patients with OA and latex-positive or latex-negative RA, JRA and from normal individuals. Selected eluates containing a mixture of IgG and IgM antigammaglobulins were separated by gel filtration chromatography on Sephadex G-200 using pH 4.5, 0.1 M sodium acetate buffer [22]. Fractions containing either IgG or IgM were concentrated by negative pressure dialysis dialyzed versus PBS and analyzed by radial immunodiffusion, precipitation, latex fixation and complement fixation.

Latex fixation tests were performed at 37 °C [10] for 1 h or at 4 °C for 18 h.

E. Precipitin Reactions

Isolated IgG or IgM rheumatoid factors were incubated with increasing amounts of reduced and alkylated aggregated γ-globulin (RAAGG). After incubation for 1 h at 37 °C and 18 h at 4 °C the precipitates were washed with cold PBS and the protein content measured by the Folin method [14].

F. Complement Fixation

The ability of IgG and IgM antigammaglobulins to fix complement with RAAGG was determined, as described previously, utilizing whole normal human serum as a source of complement [30]. Varying amounts of IgG or IgM antigammaglobulins were incubated with RAAGG at the equivalence ratios (as determined from the precipitin curves) and in antibody excess – with 70 CH50 U/ml of complement. The percent of complement fixed was calculated as described previously [23].

III. Results

A. Specificity of the Immunoadsorbent

When tested with anti-whole antiserum, only immunoglobulins could be eluted from the immunoadsorbent. 98% of the protein content of the

eluates, as determined by the Folin reaction, consisted of immunoglobulins. The supernatant from latex positive rheumatoid sera after being reacted with immunoadsorbent, generally became latex negative. The immunoadsorbent was stable with the use of acidic or high molarity buffers [16].

B. Serum Antigammaglobulin Levels

IgM antigammaglobulins were found in the majority of patients with latex-positive RA and JRA and only occasionally in latex-negative RA patients, OA patients and normals. IgG and IgA antigammaglobulins were detected in the vast majority of patients with either latex-positive RA or JRA patients, with OA, or normals. The mean concentrations of serum antigammaglobulins are given in table I. There was a considerable range of values within each group for IgG and IgA antigammaglobulins ranging from undetectable to 416 μg/ml. Despite the evident overlap of the ranges for the six groups of patients, significant differences between mean values were noted. The mean value for IgA antigammaglobulins for RA patients was significantly greater than the mean values of either the normal or OA patients. The mean value for IgA antigammaglobulins for latex-negative RA patients was significantly greater than the means for normal or OA patients and the mean value for latex-positive RA patients was significantly greater than the mean of normals, OA and latex-negative RA patients. The mean value for

Table I. Serum antigammaglobulins

	Number of patients	Antigammaglobulin, mean μg/ml ± 1 SD		
		IgG	IgA	IgM
Normals	50	98±19	52±11	<20
Osteoarthritis	49	92±16	58±10	<20
Rheumatoid arthritis				
Latex-negative	65	113±21	77± 8	<20
Latex-positive	78	210±43	102±16	139±28
Juvenile rheumatoid arthritis				
Latex-negative	77	140±20	70± 8	<20
Latex-positive	15	182±44	98±18	100±30

IgG antigammaglobulins in latex-positive RA patients was significantly greater than the mean values of the normal, OA, or latex-negative RA patients. The mean value for IgG antigammaglobulins was greater in RA patients than in either the normal or OA patients. Similarly, the mean level of IgG antigammaglobulin was greater in patients with JRA than in the normal group; levels of IgG antigammaglobulins were greater in patients with latex-positive JRA than among normals or latex-negative JRA.

Elevated levels of antigammaglobulins in any single class of immunoglobulins was usually associated with high levels of antigammaglobulins in the other two classes. IgG, IgA and IgM antigammaglobulins in the serum of patients with RA were each associated with a high frequency of subcutaneous nodules ($p<0.01$), vasculitis, poor functional class ($p<0.01$), high sedimentation index ($p<0.05$), increase in synovial fluid white blood cell count ($p<0.01$), and a large number of clinically involved inflammed joints ($p<0.01$). Relatively high levels of IgG and IgM but not IgA antigammaglobulins in the serum of RA patients were associated with radiological evidence of erosions, joint space narrowing, or bony destruction ($p<0.05$). Increasing levels of IgG and IgM antigammaglobulins in the serum ($p<0.05$) correlated with decreasing levels of serum complement. Furthermore, elevated levels of IgM antigammaglobulins in the serum of RA patients was associated with the frequent administration of antimalarial drugs and/or gold salts ($p<0.05$). There was no correlation between antigammaglobulin levels and latex fixation titers in the serum.

Elevated levels of IgM antigammaglobulins in the serum of patients with JRA was associated with a poor functional class and stage, radiological evidence of joint involvement, subcutaneous nodules, unfavorable course, antinuclear antibodies, relative decrease of serum complement levels and elevations of IgG and IgA antigammaglobulins in the serum. IgG, IgA and IgM antigammaglobulin levels in the serum were similar in latex-positive JRA patients with active or inactive disease. By contrast, significant elevations of IgG and IgA antigammaglobulins were observed in the serum of latex-negative JRA patients with active disease as compared to latex-negative JRA patients with inactive disease ($p<0.05$).

C. Synovial Fluid Antigammaglobulins

The mean values and standard deviations for antigammaglobulins of the IgG, IgA and IgM classes of antigammaglobulins found in synovial

Table II. Synovial fluid antigammaglobulins and complement levels

	Number of patients	Antigammaglobulins mean μg/ml ± 1 SD			Mean CH_{50} U/ml
		IgG	IgA	IgM	
Osteoarthritis	18	75± 18	58±31	<20	60
Rheumatoid arthritis					
Latex-negative	24	143± 27	83±19	<20	95
Latex-positive	28	163±105	83±13	99±34	45
Juvenile rheumatoid arthritis					
Latex-negative	13	171	85	<20	99
Latex-positive	3	180	91	104	33

fluid is given in table II. IgM antigammaglobulins were found predominantly in synovial fluids of patients with either latex-positive RA or JRA. IgG antigammaglobulins were detected in 95% of OA synovial fluids and/or RA and JRA synovial fluids. IgA antigammaglobulins were found in 61% of OA fluids and all RA and JRA synovial fluids. Although there was a considerable range of values of IgA and IgG antigammaglobulins in the synovial fluids (less than 20, up to 1,260 μg/ml) the mean level for IgG antigammaglobulins was greater in synovial fluids from RA patients than in those from OA patients ($p<0.05$). The mean levels of IgA antigammaglobulins in synovial fluid did not differ between patients with OA, RA and JRA.

A linear relationship existed between elevated levels of serum or synovial IgM antigammaglobulins and depressed synovial fluid CH50 complement levels in patients with RA ($p<0.01$). There was a similar, but less striking relationship, between elevated synovial fluid IgG antigammaglobulins and depressed synovial fluid complement levels ($p=0.09$). Similar trends showing an inverse correlation of synovial complement and synovial fluid IgG antigammaglobulin levels were observed in JRA.

D. Latex Fixation Tests

Isolated IgM antigammaglobulin, whether derived from patients with RA or JRA, gave a positive latex fixation test at both 37 and 4 °C with similar

Table III

	Fraction	Maximum latex fixation		Maximum complement fixed, %
		37 °C	4 °C	
Normals	IgG	0	1:80	0
Osteoarthritis	IgG	0	1:80	35
Rheumatoid arthritis				
Latex-positive	IgG	1:320	1:640	43
	IgM	1:640	1:640	58
Latex-negative	IgG	0	1:80	66
Juvenile rheumatoid arthritis				
Latex-positive	IgG	0	1:160	40
	IgM	1:2,560	1:2,560	59
Latex-negative	IgG	0	1:160	68

titers. Isolated IgG antigammaglobulins, isolated from either normal individuals or patients with OA or patients with latex-negative or positive JRA or RA, gave a positive latex fixation test at 4 °C but not at 37 °C – with one exception (table III).

E. Precipitin Reactions

Typical precipitin curves with equivalence zones were detected for all isolated IgG and IgM antigammaglobulins. The equivalence zones varied from sample to sample and the antigen to antibody ratio varied considerably for both IgG and IgM antigammaglobulins.

F. IgG Subclasses

All of the isolated IgG antigammaglobulins contained IgG1 and IgG2 and most of them contained IgG3 and some IgG4. The predominant IgG subclass was clearly IgG1. The distribution of the IgG subclasses in the isolated IgG antigammaglobulins was similar to the distribution of IgG subclasses in the serum although there was considerable individual variation.

G. Complement Fixation

Isolated IgG and IgM antigammaglobulins by themselves fixed very little, if any, human complement. However, IgM antigammaglobulins when reacted with reduced and alkylated aggregated γ-globulin at equivalence or in antibody excess, fixed up to 59% of added complement. Isolated IgG antigammaglobulins from patients with RA, JRA and one OA fixed anywhere from 5 to 69% of added complement. By contrast, three IgG antigammaglobulins derived from normal sera and one OA did not fix complement over a wide range of protein concentrations. On a molar basis, IgM antigammaglobulins were approximately ten times as efficient as IgG antigammaglobulins in fixing equivalent amounts of complement.

IV. Discussion

Rheumatoid factors have been defined as 19S IgM antibodies to γ-G globulin [6]. Rheumatoid factors were first noted in the majority of patients with RA [17]. It is now known that rheumatoid factors or antibodies to γ-globulin are not unique to RA, having been detected in many other disease states and in normal individuals [12].

Antigammaglobulins of the IgM class, however, have been observed mostly in patients with latex-positive RA [6] but also occasionally in normal individuals when a very sensitive radioimmunoassay was utilized [7]. Through the use of a number of highly sensitive immunoadsorbent techniques it has become apparent that antigammaglobulins of the IgG and IgA classes can be detected not only in many patients with rheumatic diseases but in many normals as well [26–28]. The levels, however, of the IgG and IgA antigammaglobulins tend to be somewhat higher in patients with rheumatic diseases than in patients with OA or normals [16]. These observations suggest that serum antigammaglobulins of all immunoglobulin classes may be elevated together as part of a generalized host immune response in patients with severe RA. The observations that rheumatoid factors or elevated titers of IgM or IgG antigammaglobulins correlated with depressed serum and/or synovial fluid complement levels stimulated us to examine the biologic behavior of isolated IgM and IgG antigammaglobulins. While they gave similar precipitin curves, they differed markedly in the latex fixation test. IgM antigammaglobulins would agglutinate sensitized latex particles at both 37 and 4 °C while IgG antigammaglobulin agglutinated predominantly only

at 4 °C. Similar temperature-dependent phenomena have been noticed for other IgG and IgM antibody systems [5, 11]. Of particular interest were the observations that IgG and IgM antigammaglobulins from patients with RA, JRA and the IgG from one patient with OA fixed human complement. By contrast, the IgG antigammaglobulins from normal individuals and from one patient with OA did not fix complement. This difference in complement-fixing ability between antigammaglobulins from normal individuals and from patients with RA could reflect a difference in the binding constants, conformation, avidity, secondary aggregation, or in the IgG subclass of these IgG antigammaglobulins. However, there was no apparent difference in the IgG subclass distribution in the antigammaglobulins isolated from the different individuals.

We then proceeded to calculate the theoretical amount of complement that these antigammaglobulins could fix *in vitro* and see how this may account for the *in vivo* complement fixation. Immune complement fixing ability for IgG and IgM antigammaglobulins was approximately 1.6 CH50 U/μg of antigammaglobulin. The mean concentration of IgG and IgM antigammaglobulin of synovial fluid is 163 and 99 μg, respectively. Therefore, based upon the *in vitro* observations of complement fixation with reduced and alkylated aggregated γ-globulins, these antigammaglobulins could account for approximately 259 and 160 CH50 U of complement fixed per ml. This figure is quite similar to the difference between the mean serum complement level in patients with RA (240 CH50 U/ml) and the mean level in synovial fluid (45 CH50 U/ml). These data would, therefore, suggest that the degree of *in vitro* complement fixation between IgM and/or IgG antigammaglobulins and reduced and alkylated aggregated globulin theoretically could account, at least in part, for the *in vivo* complement depletion in rheumatoid synovial fluids. However, the present data and theoretical calculations do indeed represent static measurements and do not take into account the constant turnover of complement with the synovial space. Nor does it exclude complement fixation or depletion by some other mechanism or complexes.

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