ARTHRITIS AND RHEUMATISM
New Series Vol. 14, No. 6, Nov.-Dec. 1971. Published by Medical Department, Harper & Row, Publishers, Inc. Printed in U.S.A.

Serum and Synovial Fluid IgG, IgA and IgM Antigammaglobulins in Rheumatoid Arthritis

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Antigammaglobulins of IgG, IgA and IgM classes were measured in normal individuals and in patients with osteoarthritis or rheumatoid arthritis. Serum IgG and IgA and synovial fluid IgG antigammaglobulin levels were significantly higher in patients with rheumatoid arthritis than in other individuals, with highest levels occurring in patients with positive latex fixation tests. IgM antigammaglobulins were elevated only in patients with latex positive rheumatoid arthritis. Increased serum levels of IgG, IgA and IgM antigammaglobulins were each associated with clinical findings of severe rheumatoid arthritis. Increased levels of serum and synovial fluid IgG and IgM antigammaglobulins were each associated with diminished serum and synovial fluid complement levels.

Antigammaglobulins, or rheumatoid factors, can be demonstrated in the sera of the majority of patients with rheumatoid arthritis (RA) by the agglutination of sensitized sheep red blood cells, bentonite, or latex particles coated with gammaglobulin (1–3). These tests provide only a semiquantitative estimate of the amount of antigammaglobulins present and do not indicate the distribution of the antigammaglobulins among the different immunoglobulin classes.

Rheumatoid factors (RF) were first described as 19S γM globulins (4, 5). Later RF were recognized among lower molecular weight proteins (6–9) and as intermediate complexes (6, 7, 9). Subsequently RF were identified among γG and γA globulins (10–16).

The presence of RF, as measured by agglutination tests and presumed to represent IgM antibodies, has been associated with an unremitting disease course (17, 18), the occurrence of subcutaneous nodules (17–19), limited functional capacity (17, 18), radiologic evidence of joint destruction (18, 20), and diminished serum (20, 21) and synovial fluid (20, 22) complement levels. IgG and IgA antigammaglobulins, however, have not been clearly associated with these clinical manifestations of RA (11, 15).

The purpose of this study was to quanti-

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Supported by grants from the United States Public Health Service (Research grants AM 11414, AM 05577, AM 12051, and Training Grant AM 5076) and the Massachusetts Arthritis Foundation.

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Presented in part at the Annual Meeting of the American Rheumatism Association, June 20, 1970, Detroit, Mich.

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Submitted for publication Feb 17, 1971; accepted May 5, 1971.

fy levels of IgG, IgA and IgM antigammaglobulins in serum of normal individuals, in serum and synovial fluid from patients with osteoarthritis, and in serum and synovial fluid from patients with rheumatoid arthritis having either positive or negative latex agglutination tests for RF. The levels of these antigammaglobulins were then tested for associations with a number of clinical parameters of disease in patients with rheumatoid arthritis.

MATERIALS AND METHODS

Population

Fifty normal individuals were randomly selected from among hospital employees. Forty-nine patients with osteoarthritis (OA) and 143 with RA were selected on the basis of adequate clinical data and availability of serum or synovial fluid (SF) samples. Many patients with RA with a negative agglutination test for RF were deliberately included to render desirable sample sizes. Hereafter, RA patients with positive serum latex agglutination tests for RF will be designated as RF (+), and RA patients with negative tests will be referred to as RF (−).

Clinical Studies

Hospital records were reviewed for the patient's age, sex, race, duration of disease, presence of subcutaneous nodules, fever, weight loss and uveitis. The total number of inflamed, painful, or limited joints was counted. A hand, foot, elbow, shoulder, wrist, hip, knee, ankle, spine or temporomandibular joint was each considered as a single joint. Presence of peripheral neuropathy or vasculitic skin changes was noted. Radiologic findings of erosions, joint narrowing, degenerative changes, ankylosis, dislocation, or subluxation were recorded. Use of salicylates, antimalarial drugs, gold salts, indomethacin, phenylbutazone, propoxyphene, or corticosteroids was tabulated. The number of criteria considered diagnostic for RA was counted (23). Patients were evaluated for functional capacity according to the criteria of Lansbury (24). Disease course was characterized as remitting or unremitting as described by Sharp *et al* (17). Patients having systemic lupus erythematosus, Reiter's syndrome, juvenile rheumatoid arthritis, ankylosing spondylitis, gout, inflammatory bowel disease, polymyositis, progressive systemic sclerosis, or chronic liver disease were excluded from the study.

Laboratory Studies

The following laboratory studies were performed: complete blood count, blood sedimentation index (25), serum protein electrophoresis, LE cell preparation, antinuclear antibody test (26), serum and synovial fluid whole hemolytic complement (CH_{50}) levels (27), latex fixation test (LFT) (28), and synovial fluid analysis for white blood cells (WBC), protein and mucin clot formation (29).

Immunologic Studies

Antigammaglobulins in serum and SF were adsorbed to and eluted from insoluble human IgG (Fig. 1). Human IgG was prepared by ammonium sulfate fractionation of Cohn fraction II*. Purity of the preparation was verified by immunoelectrophoresis (30) and double diffusion in agar (31) against appropriate antisera. The IgG was insolubilized with glutaraldehyde, washed and homogenized, according to the method of Avrameas and Ternynck (32). Aliquots of homogenized insoluble material were resolubilized by incubation in 1N NaOH for two hours at 56 C so that protein content could be determined by the Folin method (33).

Adsorption of specimens and elution of antibodies were performed by a modification of the techniques described by Torrigiani *et al* (11, 12, 15) and Avrameas and Ternynck (32) (Fig 1). Twenty-milligram aliquots of insoluble IgG were incubated with 0.25 ml of serum (or SF) at 37 C for one hour and at 4 C overnight. The suspension was centrifuged at 4 C for 15 minutes at 1500 *g*, and the supernatants were saved for further study. The immunoadsorbent was washed four times with cold 0.01M phosphate-buffered saline, pH 7.4 (PBS). Final washes had optical densities of less than 0.050 at 280 mμ. Antigammaglobulins were then eluted by incubating the immunoadsorbent with 0.10M glycine-HCl buffer, pH 2.8, for 45 minutes at 4 C, followed by centrifugation for 15 minutes at 6000 *g* at 4 C. Three elutions were required to recover the adsorbed protein. Eluates were brought to pH 7.4 by titration with 0.10N NaOH, passed through 0.45 μ Millipore® filters, and concentrated back to the original sample volume by negative pressure dialysis.

*Kind gift of Lederle Laboratories, Pearl River, NY.

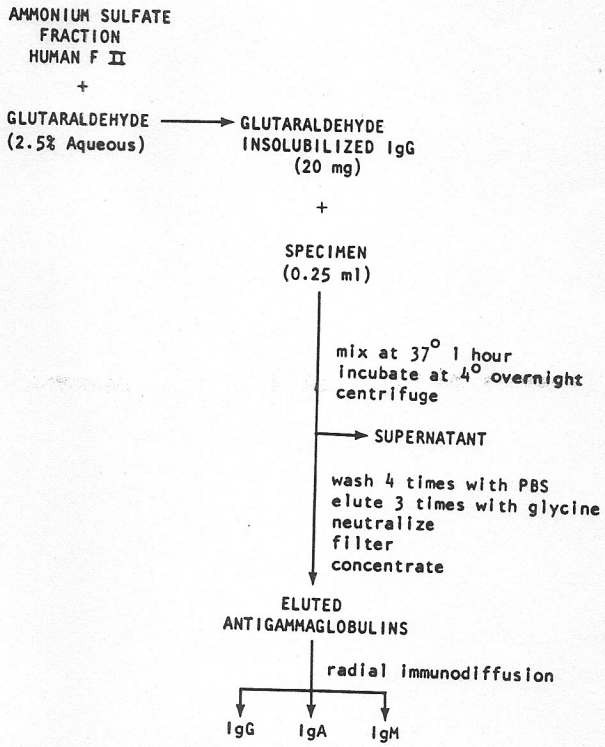

Fig 1. Method of measuring antigammaglobulins using a human IgG immunoadsorbent.

Protein content in eluates was measured by the Folin method (33). Immunoelectrophoresis (30) of eluates was performed with antisera to individual proteins and to whole human serum. IgG, IgA, IgM and human serum albumin (HSA) in eluates were determined by radial immunodiffusion (34). As little as 10 μg/ml of IgG and IgA, 20 μg/ml of IgM, and 5 μg/ml of HSA could be detected. These antigammaglobulin measurements were reproducible to within 4–8% standard error of the mean.

IgG, IgA and IgM antigammaglobulins were isolated in large amounts from several hundred milliliters of sera from patients with RF(+) RA, RF(−) RA, OA and from a normal individual. IgM was separated by chromatography with Sephadex G200 (35). IgG and IgA were isolated by chromatography with diethylaminoethyl cellulose or carboxymethyl cellulose (36).

Data Analysis

Standard statistical methods were employed to analyze the data (37). Many computations were performed with the assistance of computer programs; *t* tests and chi-square tests were performed, as appropriate to the data, and *P* values were calculated. *P* values less than 0.05 were considered significant—that is, indicating differences unlikely to represent mere chance occurrences. The coefficient of correlation, *r*, reflecting the linear association between two variables, was determined in certain instances and *P* values ascertained.

RESULTS

Specificity of the Immunoadsorbent

The IgG which was subsequently insolubilized contained IgG_1, IgG_2, IgG_3 and IgG_4 in the proportions found in normal sera. No other serum proteins were present in the ammonium sulfate fraction, as tested by double diffusion in agar and immunoelectrophoresis against antisera to whole human serum, IgA and IgM. When the insol-

uble IgG immunoadsorbent was reacted with a rabbit antiserum to whole human serum, only antibodies to nonimmunoglobulins remained in the supernatant; only antibodies to IgG could be eluted from the immunoadsorbent. When eluates from patients' sera were analyzed, 98% of the protein content of the eluates (determined by the Folin reaction) consisted of immunoglobulins (determined by immunoelectrophoresis and radial immunodiffusion). Clq protein was detected in concentrations of less than 20 μg/ml in some eluates by radial immunodiffusion with specific antiserum. Not more than 5 μg/ml of HSA could be detected in eluates.

The supernatants from latex-positive RA sera, which had been reacted with the immunoadsorbent, generally became latex negative. The eluates derived from latex-positive sera were latex positive. Eluates derived from sera of normal individuals, patients with OA and patients with RF(−) RA, when concentrated several fold, were also latex positive. Isolated IgG, IgA and IgM antigammaglobulins each gave positive latex fixation tests. The IgG immunoadsorbent was stable under experimental conditions. When samples of immunoadsorbent were incubated alone with different buffers—PBS, 0.10M glycine-HCl pH 2.8, or 0.10M sodium acetate pH 4.0—no IgG was released.

Serum Antigammaglobulin Levels

Levels of IgM antigammaglobulins are presented in Figure 2. Each point in the figure represents a determination from an individual patient's serum. Solid lines denote mean values and shaded areas indicate the 95% confidence limit for the means. With the exception of one normal, one OA and two RF(−) individuals, IgM antigammaglobulins were detected only in RF(+) patients. Their mean level was 139 ± 28 μg/ml (range: <20–888 μg/ml).

Levels of IgA antigammaglobulins are similarly shown in Figure 3. They were detected in 76% of normal individuals with a mean level of 52 ± 11 μg/ml (range: <20–132 μg/ml), in 95% of OA patients with a mean level of 58 ± 10 μg/ml (range: <20–146 μg/ml), in 95% of RF(−) RA patients with a mean level of 77 ± 8 μg/ml

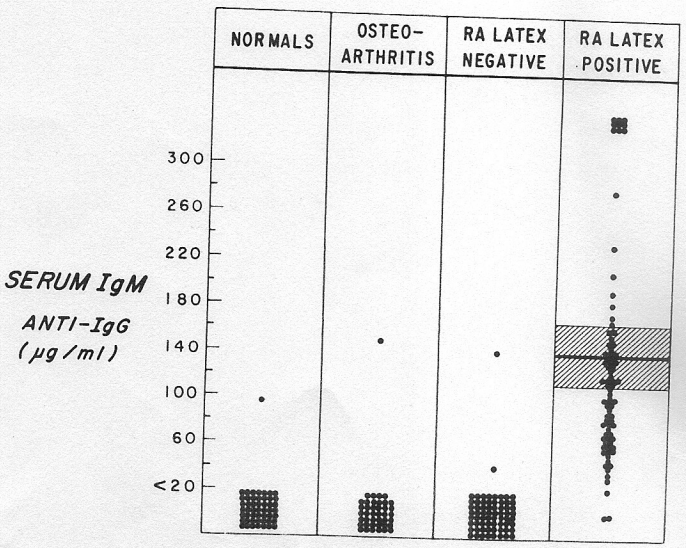

Fig 2. Serum IgM antigammaglobulin levels in normal individuals, patients with osteoarthritis, latex-negative rheumatoid arthritis (RA), and latex-positive RA. Each point represents a determination from an individual patient's serum. Solid lines denote mean values for each group, and shaded areas indicate the 95% confidence limit for the means.

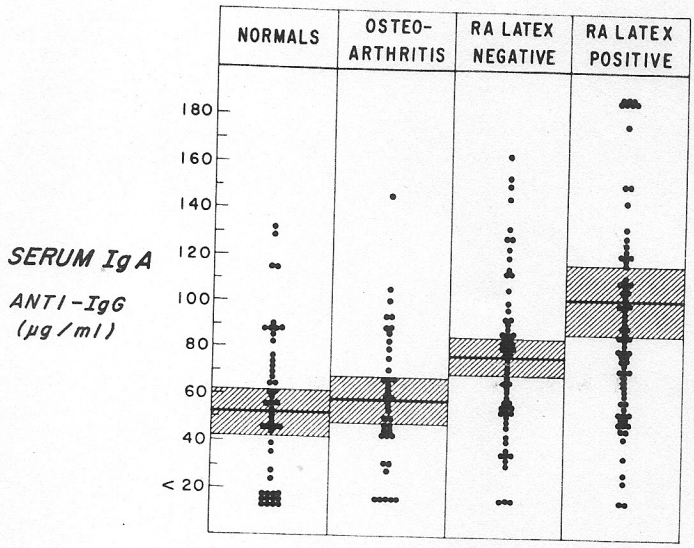

Fig 3. Serum IgA antigammaglobulin levels in normal individuals, patients with osteoarthritis, latex-negative rheumatoid arthritis (RA) and latex-positive RA. Each point represents a determination from an individual patient's serum. Solid lines denote mean values for each group, and shaded areas indicate the 95% confidence limit for the means.

(range: <20–163 μg/ml) and in 97% of RF(+) RA patients with a mean level of 102 ± 16 μg/ml (range: <20–416 μg/ml). Despite the evident overlap of the ranges for the four groups, significant differences between their mean values existed. The mean value for all RA patients (89 μg/ml) was significantly greater than the mean value for either the normal or OA patients ($P<0.05$). Similarly the mean value for RF(−) RA patients was significantly greater than that for normal or OA patients ($P<0.05$). The mean value for RF(+) RA patients was significantly elevated above the means for any of the other three groups ($P<0.05$).

Serum IgG antigammaglobulin levels are depicted in figure 4. They were detected in

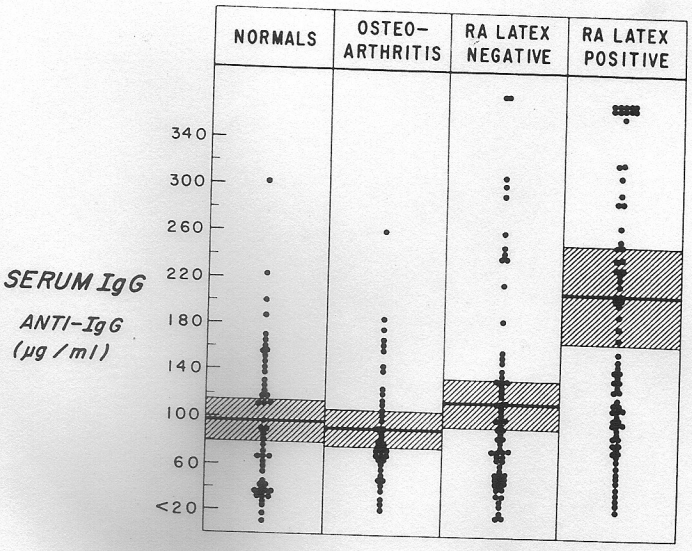

Fig 4. Serum IgG antigammaglobulin levels in normal individuals, patients with osteoarthritis, latex-negative rheumatoid arthritis (RA) and latex-positive RA. Each point represents a determination from an individual patient's serum. Solid lines denote mean values for each group, and shaded areas indicate the 95% confidence limit for the means.

all individuals studied. Mean levels were 98 ± 19 μg/ml (range: 16–300 μg/ml) for normal individuals, 92 ± 16 μg/ml (range: 25–259 μg/ml) for patients with OA, 113 ± 21 μg/ml (range: 16–400 μg/ml) for RF(−) RA patients, and 210 ± 43 μg/ml (range: 22–864 μg/ml) for RF(+) RA patients. The mean value for all RA patients (157 μg/ml) was significantly greater than for either normal or OA groups ($P<0.05$). The mean value for RF(+) RA patients was significantly greater than the mean values for either normal, OA, or RF(−) RA patients ($P<0.05$).

An elevated serum antigammaglobulin value in any single class of immunoglobulins was usually associated with high levels of serum antigammaglobulins in the other two immunoglobulin classes ($P<0.0001$) (Table 1). Occasional patients were observed with elevation of serum antigammaglobulins of only one immunoglobulin class (IgG or IgA or IgM) and "normal" levels of antigammaglobulins of the other two immunoglobulin classes. These patients tended to portray a clinical picture similar to patients with elevation of serum antigammaglobulins of all three classes of immunoglobulins (Table 1).

Relative elevations of serum IgG, IgA and IgM antigammaglobulin levels in patients with rheumatoid arthritis were each associated with a high frequency of subcutaneous nodules ($P<0.01$), vasculitis, poor ARA functional classification ($P<0.01$), high sedimentation index ($P<0.05$), increased SF

Table 1. Associations of Serum Antigammaglobulins in Rheumatoid Arthritis

Parameter	Antigammaglobulin levels					
	IgG		IgA		IgM	
	Statistical test	P	Statistical test	P	Statistical test	P
Serum IgG antigammaglobulin level	—	—	$r = 0.642$	0.01	$r = 0.491$	0.01
Serum IgA antigammaglobulin level	$r = 0.642$	0.01	—	—	$r = 0.450$	0.01
Serum IgM antigammaglobulin level	$r = 0.491$	0.01	$r = 0.450$	0.01	—	—
Presence of subcutaneous nodules	$t = 3.15$	0.01	$t = 3.20$	0.01	$t = 3.54$	0.01
ARA functional classification	$r = 0.182$	0.01	$r = 0.220$	0.01	$r = 0.287$	0.01
Blood sedimentation index	$r = 0.302$	0.01	$r = 0.369$	0.01	$r = 0.209$	0.05
Synovial fluid WBC/cu mm	$r = 0.384$	0.01	$r = 0.515$	0.01	$r = 0.386$	0.01
Number of inflamed joints	$r = 0.242$	0.01	$r = 0.344$	0.01	$r = 0.318$	0.01
Number of joints limited in motion	$r = 0.264$	0.01	$r = 0.325$	0.01	$r = 0.227$	0.01
Presence of radiological erosions	$t = 2.73$	0.05	$t = 1.20$	NS	$t = 2.45$	0.05
Presence of radiological narrowing	$t = 2.23$	0.05	$t = 0.62$	NS	$t = 2.37$	0.05
Presence of radiological bone destruction	$t = 2.41$	0.05	$t = 1.75$	NS	$t = 2.14$	0.05
Serum CH_{50}	$r = -0.207$	0.05	$r = -0.108$	NS	$r = -0.214$	0.05
Synovial fluid protein content	$r = 0.035$	NS	$r = 0.130$	NS	$r = -0.336$	0.05
Use of antimalarial drugs	$t = 1.29$	NS	$t = 1.30$	NS	$t = 2.71$	0.05
Administration of gold salts	$t = 0.47$	NS	$t = 0.50$	NS	$t = 3.32$	0.01
Synovial fluid IgG antigammaglobulin level	$r = 0.137$	NS	$r = 0.084$	NS	$r = 0.119$	NS
Synovial fluid IgA antigammaglobulin level	$r = 0.048$	NS	$r = 0.185$	NS	$r = -0.085$	NS
Synovial fluid IgM antigammaglobulin level	$r = 0.250$	NS	$r = 0.084$	NS	$r = 0.536$	0.01

* $P < 0.05$
† $P < 0.01$
NS = not significant

cell count ($P<0.01$) and a large number of joints that were clinically inflamed or limited in motion ($P<0.01$). Comparatively high levels of serum IgG and IgM, but not IgA, antigammaglobulins were associated with radiologic changes of juxta-articular erosions, cartilaginous narrowing or bony destruction ($P<0.05$). Increasing serum levels of IgG ($P<0.05$) and IgM ($P<0.05$) antigammaglobulins correlated with decreasing levels of serum complement. Increased serum IgM antigammaglobulin levels, in addition, were associated with decreased SF protein levels ($P<0.05$), frequent use of antimalarial drugs ($P<0.05$) and the administration of gold salts ($P<0.01$). The remaining clinical parameters assessed were unrelated to serum antigammaglobulin levels (Table 1).

Although, as indicated, RF(+) patients with RA had higher levels of serum antigammaglobulins than did RF(−) patients, no linear relationship existed between LFT titers and serum antigammaglobulin levels.

Synovial Fluid Antigammaglobulins

The mean values for SF antigammaglobulins of IgG, IgA and IgM classes for 18 OA patients, 24 RF(−) and 28 RF(+) RA patients are listed in Table 2.

IgG antigammaglobulins were detected in 95% of OA SFs with a mean level of 75 ± 18 μg/ml (range: <20–144 μg/ml). They were found in all RA fluids, with a mean level of 143 ± 27 μg/ml (range: 26–450 μg/ml) for RF(−) patients, and 163 ±105 μg/ml (range: 31–1260 μg/ml) for RF(+) patients. Although the levels for RF(−) and RF(+) RA patients were similar, the mean level for all RA patients (157 μg/ml) was significantly greater than that for OA patients ($P<0.05$).

IgA antigammaglobulins were detected in 61% of OA fluids with a mean level of 58 ± 31 μg/ml (range: <20–132 μg/ml), in 80% of RF(−) RA fluids with a mean level of 83 ± 19 μg/ml (range: <20–180 μg/ml) and in 90% of RF(+) fluids with a mean level of 83 ± 13 μg/ml (range: <20–144 μg/ml). None of these mean levels was significantly different from another.

IgM antigammaglobulins were detected in 61% of RF(+) individuals with a mean value of 99 ± 34 μg/ml (range: <20–288 μg/ml), but only in 8% of RF(−) RA patients. They were not found in OA fluids.

Levels of SF and serum IgM antigammaglobulins correlated with each other ($P<0.01$); levels of IgG and IgA antigammaglobulins did not (Table 1). Although SF IgG and IgA antigammaglobulin levels were greater than serum IgG and IgA antigammaglobulin levels in patients with RF(−) RA, the differences were not significant.

Patients with RF(+) RA had lower synovial fluid CH_{50} levels than did patients with RF(−) RA. However, there was no

Table 2. Synovial Fluid Antigammaglobulin and Complement Levels

	No. of patients	Antigammaglobulin (μg/ml)*			
		γG	γA	γM	CH_{50} (μ/ml)
Osteoarthritis	18	75	58	<20	60
Rheumatoid arthritis					
Latex-negative	24	143	83	<20	95
Latex-positive	28	163	83	99	45

* Mean values

linear relationship between the CH_{50} level and LFT titer. A linear relationship did exist between elevated serum ($r = -0.342$; $P<0.01$) or SF ($r = -0.501$; $P<0.01$) IgM antigammaglobulin levels and depressed SF CH_{50} values in patients with RA. There was a similar, but slightly less striking, relationship between elevated SF IgG antigammaglobulin and lowered SF CH_{50} levels ($r=-0.210$; $P=0.09$). Correction of SF antigammaglobulin or complement measurements for SF total protein content did not affect these results.

DISCUSSION

Antigammaglobulins were isolated from sera of normal individuals, patients with rheumatoid arthritis or osteoarthritis, and from the synovial fluid of patients with rheumatoid arthritis or osteoarthritis. The IgG, IgA, and IgM content of these antigammaglobulins was determined.

The antigammaglobulins were adsorbed to and then eluted from glutaraldehyde-insolubilized human IgG. Avrameas and Ternynck (32) have shown that only specific antibodies combine with glutaraldehyde insolubilized antigens. They incubated normal rabbit sera, human or rabbit IgG and albumin with insolubilized albumin, IgG, Bence-Jones proteins and with other insoluble derivatives. Virtually no protein could be adsorbed to or eluted from these preparations. Conversely, insolubilized normal human serum was able to adsorb completely most antibodies when incubated with homologous rabbit or horse antisera. In the present study, when antiserum to whole human serum was adsorbed to insoluble human IgG, only antibodies to IgG could subsequently be eluted.

Nearly all protein eluted from the immunoadsorbent in the current study consisted of immunoglobulins. When sera from normal individuals, patients with OA, RF(−) RA and RF(+) RA were incubated with insoluble human IgG, material with latex agglutinating ability was eluted in each instance. Furthermore, it was shown that isolated preparations of IgG, IgA and IgM antigammaglobulins each possessed latex agglutinating activity.

It is known that antigammaglobulins are not unique to RA, having been detected in other disease states and in normal individuals (38). IgM antigammaglobulins have been observed in most patients with RF(+) RA (4–6, 8–11, 39) and have also been seen in occasional normal individuals, using a radioimmunoassay for the detection of IgM RF (39). IgG and IgA antigammaglobulins have been described in the sera of patients with RA and in normal individuals, using bisdiazotized-benzidene aggregated IgG as an immunoadsorbe ., 15), by using solid, heat-denatured FII as immunoadsorbent (10), the technic of hemagglutination inhibition (13) and by using a bromoacetylcellulose human IgG immunoadsorbent in the serum of a patient with systemic lupus erythematosus and hyperglobulinemic purpura (16). IgA antigammaglobulin has also been identified in the urine of patients with RA (14).

In this study, IgG and IgA antigammaglobulins could be detected at low levels in sera from normal individuals and patients with OA. IgG, IgA and IgM antigammaglobulins could all be measured at significantly higher levels in sera from patients with RA. The antigammaglobulin levels in this study are somewhat higher than those reported by Torrigiani *et al* (11, 15). These differences may be due to the greater binding affinity of RF for autologous IgG, used in this study, than for the animal gammaglobulin used by Torrigiani *et al* (40–42). Levels of IgM antigammaglobulins reported here are comparable

to those reported by radioimmunoassay determinations with human IgG used as the antigen (39).

Serum levels of these three antigammaglobulins were elevated together and were associated with changes of severe illness in patients with RA. Although it has been recognized that extensive disease correlated with the presence of RF, as measured by agglutination tests, the elevated levels of IgG and IgA as well as IgM antigammaglobulins in these patients' sera have not been appreciated. These observations suggest that serum antigammaglobulins of all these immunoglobulin classes may be elevated together as part of a generalized host immune response in severe rheumatoid arthritis. In the present study, there was no apparent relationship between latex fixation titers for RF and serum or SF levels of IgG or IgM antigammaglobulins. This discrepancy may be due, in part, to the high variability in agglutination titers which will result from similar concentrations of antibodies (43).

Synovial fluid antigammaglobulins of IgG, IgA and IgM classes were detected and measured in synovial fluids from patients with osteoarthritis or rheumatoid arthritis. IgG and IgM antigammaglobulin levels were increased in fluids from patients with RA as compared to patients with OA. Levels of both of these antigammaglobulins, particularly IgM, correlated inversely with synovial fluid complement levels.

It is known that patients with RA and a positive test for rheumatoid factor have more severe disease than do patients with a negative test (17–22), and that these seropositive patients may have diminished serum (20, 21, 44) and synovial fluid (20, 22, 45–47) complement levels. Sera containing rheumatoid factor fix complement with heat-aggregated, reduced and alkylated IgG (48). Highly purified IgM RF can increase the complement–fixing ability of soluble immune complexes (49). Winchester *et al* have also described an association between low synovial fluid complement levels and the presence of abundant IgG-anti-IgG complexes in joint fluids from a group of patients with RA, predominantly seropositive (45). These studies suggest that IgG and IgM rheumatoid factors participate in the immunologic activation of the complement system in patients with RA, and that activation of the complement sequence occurs intra-articularly and causes the inflammatory changes observed pathologically and radiologically (20, 22).

However, even though rheumatoid factors appear to fix complement with aggregates of gammaglobulin, we were unable to confirm the linear relationship between serum or synovial fluid complement levels and latex fixation agglutination titers demonstrated by Hedberg (46). There was a linear relationship, however, between elevated serum or synovial fluid IgG and IgM antigammaglobulin levels and lowered serum or synovial fluid complement levels. These observations lend further support to the hypothesis that hypocomplementemia in patients with RA is due, at least in part, to fixation by antigammaglobulins (22, 43, 45). In addition, these studies suggest that more biologic information might be gained about antigammaglobulins, or other antibodies, by quantitative measurements rather than by agglutination titers.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of Mrs. Mary Megson, and the generous and expert advice of Mr. Raymond Neff, Department of Biostatistics, Harvard School of Public Health.

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