

Antibodies to bovine gamma globulin (BGG) and the occurrence of a BGG-like substance in systemic lupus erythematosus sera

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The possibility that ingested antigens may play a role in the pathogenesis of systemic lupus erythematosus (SLE) has not been previously studied. Because this might be of considerable importance, a study was undertaken to examine the occurrence of antibodies to bovine gamma globulin (BGG) and bovine serum albumin (BSA) in SLE sera. In addition, an assay for the detection of trace amounts of BGG in human serum was developed. Antibodies to BGG were found in over 60 per cent of SLE sera and in 40 per cent of normal sera. Fifty per cent of normal sera and 40 per cent of SLE sera bound significant amounts of BSA. However, there was a prevalence of higher levels of antibody to both antigens in SLE. Although this may simply represent another example of hyperreactivity in SLE, both BGG and BSA are commonly ingested proteins. If antigenically intact molecules enter the circulation of certain patients, potentially pathogenic immune complexes might be formed. This possibility is supported by the finding of a BGG-like material in at least two sera, and in one case the coexistence of this material and antibodies to BGG was demonstrated. Since exposure to ingested antigens is a continual occurrence, the potential role of a wide variety of food antigens in the immune complex diseases should be carefully evaluated.

Patients with systemic lupus erythematosus (SLE) have antibodies which react with a wide variety of antigens such as deoxyribonucleic acid (DNA), soluble nucleoprotein, red cells, white cells, and clotting factors.² These antibodies may be instrumental in producing disease manifestations, either directly, as in the case of the hemolytic anemia resulting from red cell antibodies,³ or indirectly, as in the immune complex type of nephritis.⁴ Considerable information is available regarding antibodies to DNA, and it appears that soluble DNA-anti-DNA complexes may be formed in the sera of certain patients, setting off a chain of events resulting in renal disease.^{5, 6}

It is not as yet known whether the immunogen responsible for anti-DNA

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antibodies in SLE is endogenous or from an exogenous source, such as a viral or bacterial nucleic acid. Although the main studies on the pathogenesis of SLE nephritis have been directed toward antigen-antibody complexes containing endogenously available antigen, it is known that immune complex disease in both human beings and experimental animals can also occur due to the interaction of antibodies with exogenous antigen. Examples are the classic serum sickness in man developing after the injection of horse tetanus antitoxin⁷ and the nephritis in rabbits developing after the injections of BSA or BGG.^{8, 9}

Certain human sera are known to contain antibodies to BGG and BSA, presumably as a consequence of the ingestion of milk and beef.^{10, 11} Furthermore, antigenically intact BSA has previously been demonstrated in the sera of premature infants¹² and rabbits fed BSA in their drinking water.¹³ This paper reports studies showing an increased incidence of antibodies to bovine IgG in the sera of patients with SLE. In addition, there is a trend toward higher titers of antibodies to BSA in SLE sera than in normal sera. If the ingestion of milk or beef leads to the absorption of antigenically intact bovine proteins in adult human subjects, potentially pathogenic immune complexes could be formed in the circulation of certain patients with BGG and/or BSA antibodies. Of considerable interest in this regard are two sera which contained a BGG-like material, especially since one also contained anti-BGG.

MATERIALS AND METHODS

Sera

Patients with SLE and rheumatoid arthritis (RA) were seen and studied at the Rockefeller University Hospital, New York, New York, and the National Jewish Hospital, Denver, Colorado. Sera were obtained from normal laboratory and hospital personnel and blood bank donors, the age and sex distribution of whom were similar to those of the patient population. Sera were also obtained from rabbits immunized with BGG or bovine IgG Fab in complete Freund's adjuvant.

Antigens

Bovine serum was obtained from Microbiological Associates (Bethesda, Maryland). Human Fraction II (Fr II) was purchased from Lederle Laboratories (Pearl River, New York), and bovine, sheep, porcine, chicken, dog, rabbit, horse, and rat Fr II's were obtained from Pentex Biochemicals (Kankakee, Illinois). All the Fr II's were further purified by diethylaminoethyl (DEAE)-cellulose chromatography in 0.01M phosphate buffer, pH 8.0.¹⁴ Crystalline bovine plasma albumin was obtained from the Armour Company (Chicago, Illinois) and pasteurized skim milk from a local dairy.

Extracts of raw, rare, and well-done ground beef were prepared by adding 40 ml. of physiologic saline buffered with 0.01M phosphate (PBS), pH 7.0, to 20 Gm. samples of beef and by homogenizing for 2 minutes in a Sorvall homogenizer.

Preparation of Bovine Fab

¹³¹I-bovine IgG Fab was used as a test antigen in the ammonium sulfate test,^{15, 16} since intact BGG is insoluble in half-saturated ammonium sulfate (50 per cent SAS). Bovine Fr II was fractionated on a DEAE-cellulose column equilibrated with 0.02M sodium phosphate buffer, pH 8.0. The peak eluting with the starting buffer was pooled and digested with papain (Worthington Biochemicals, Freehold, New Jersey) for 24 hours at 37° C.¹⁷ The digest was dialyzed for 24 hours against 0.02M sodium phosphate buffer, pH 8.0, and then chromatographed on a DEAE-cellulose column equilibrated with the same buffer to separate the Fab

TABLE I. Precipitins against bovine serum by immunodiffusion

Sera	Positive	Negative	Per cent positive
Normal	9	319	3
SLE	13	76	15

from the Fc fragment. To minimize the number of molecules insoluble in 50 per cent SAS, the Fab fraction was pooled, an equal volume of cold saturated ammonium sulfate (SAS) was added, and the mixture was incubated 30 minutes at 4° C. The precipitate was removed by centrifugation and the supernatant dialyzed against PBS for 4 days at 4° C. to remove ammonium sulfate. The solution was chromatographed on a Sephadex G-200 column equilibrated with PBS. The Fab peak was pooled and concentrated. Immuno-electrophoretic analysis showed no contamination with intact IgG or Fc fragments.

The proteins were labeled with ^{131}I by the chloramine-T method.¹⁸

Immunologic techniques

Precipitin reactions were demonstrated by double diffusion in 0.6 per cent agarose (Seakem, Bausch and Lomb, Rochester, New York) in PBS. In the initial screen for antibody, sera were tested against six dilutions of bovine serum (1:10 to 1:5000). For the detection of antigen in the human sera, diluted rabbit anti-BGG capable of giving an observable precipitin line with as little as 1 μg of BGG per milliliter was used. Immuno-electrophoresis (IEP) was carried out as described previously,¹⁹ but with one per cent agarose in pH 8.6 barbital buffer. Hemagglutination assays were performed with a microtiter apparatus (Cooke Engineering Company, Alexandria, Virginia), with the use of normal human O+ erythrocytes coated with bovine gamma globulin as antigen by the bis-diazotized benzidine (BDB) technique.²⁰ Rheumatoid factor was assessed by hemagglutination with erythrocytes coated with a human anti-RH serum (anti-CD Ripley).²¹

The ammonium sulfate assay was performed as follows: Nitrogen (N) ^{131}I -bovine Fab, 0.02 μg , in 0.5 ml. of 1:100 normal human serum in borate buffer, pH 8.4, was added to 0.5 ml. of a 1:10 dilution of test serum in borate buffer. After mixing, the tubes were incubated overnight at 4° C. and processed as described previously.¹⁵ Since BSA is soluble in 50 per cent SAS, intact ^{131}I -BSA was used for the ammonium sulfate technique. Results are reported as per cent of radioactivity bound, corrected for the radioactivity in the precipitate of a normal control serum.²² The ammonium sulfate inhibition assay¹³ was performed to determine the capacity of known amounts of IgG's from various species to inhibit the binding of ^{131}I -bovine Fab by SLE sera or rabbit anti-BGG sera. Antiserum (0.75 ml.) plus 0.75 ml. of inhibitor were mixed and incubated overnight at 4° C. Duplicate 0.5 ml. aliquots were removed, and 0.5 ml. containing 0.02 of N ^{131}I -Fab was added to each and the tubes incubated overnight at 4° C. The tubes were then processed as described previously.¹³ In the absence of inhibitor, the antiserum in the 0.5 ml. aliquots was capable of binding 50 per cent of the radioactive antigen. The decrease in binding in the presence of inhibitor gave a quantitative estimate of the amount of inhibitor present. Two inhibitory SLE plasmas were titered by testing serial dilutions in an inhibition assay.

Immunoglobulin quantitation was performed by radial immunodiffusion in Immuno-Plates (Hyland Laboratories, Costa Mesa, California).

RESULTS

Incidence of anti-BGG and anti-BSA in SLE and normal subjects

Sera were initially examined by double immunodiffusion against bovine serum, and, as seen in Table I, only 3 per cent of 328 normal sera had detectable precipitins to bovine serum as compared to 15 per cent of 89 SLE sera ($p < 0.001$ by χ^2 test). Of note was the marked dilution of bovine serum often necessary to obtain a positive precipitin test. In many cases precipitin lines were detectable

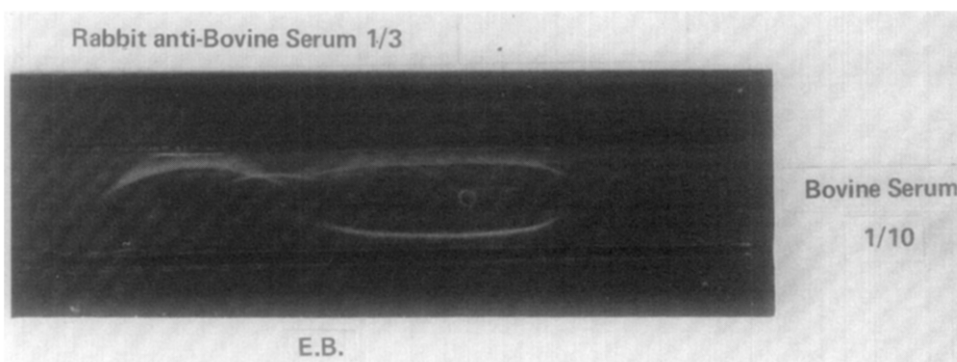


FIG. 1. Immunoelectrophoresis of bovine serum diluted 1:10 to avoid antigen excess. The trough with diluted rabbit antiserum was filled once and the trough with SLE serum from Patient E. B. 6 times. Only one line, in the bovine IgG region, is seen with the SLE serum.

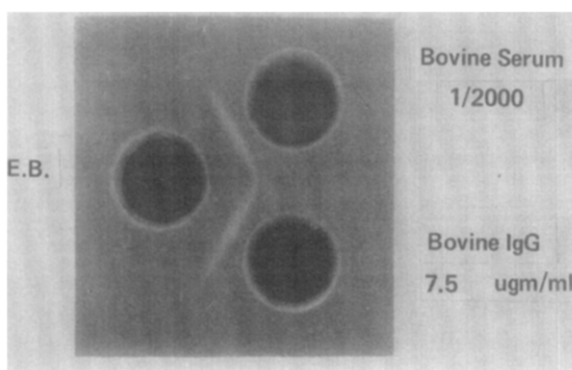


FIG. 2. Reaction of an SLE serum with bovine serum and isolated IgG. A line of identity is seen indicating the reactive antigen in the bovine serum is IgG.

only with bovine serum diluted 1:500 or more. The reacting component or components in the bovine serum were determined by IEP. Seven of the 13 SLE sera with precipitins had enough precipitating antibody to give a detectable line by IEP, and 6 reacted only with bovine IgG. The SLE serum in Fig. 1 reacted with bovine IgG₁, and the others reacted with both IgG₁ and IgG₂.²³ The seventh SLE serum reacted only with bovine IgM. Eleven of the SLE sera and all 9 of the normal sera reacted with isolated bovine IgG by immunodiffusion. In every case a line of identity with bovine serum was formed (Fig. 2), indicating the reactive bovine serum antigen was also IgG. By immunodiffusion the other 2 SLE sera reacted against IgM in the bovine serum, although one was too weak to give a precipitin line by IEP.

By use of BGG-coated erythrocytes, a large number of positive hemagglutination tests resulted, with both the normal and SLE sera, but with a prevalence of higher titers with the SLE sera. Thirteen of 65 SLE sera but only 4 of 64 normal sera had titers of 1:64 or higher. In general, the highest titer sera were those which had been positive in the precipitin tests. Hemagglutination could be completely blocked by pre-incubating the sera with bovine IgG and partially blocked by sheep IgG, but no inhibition was observed with human, rabbit, pig, or horse IgG's.

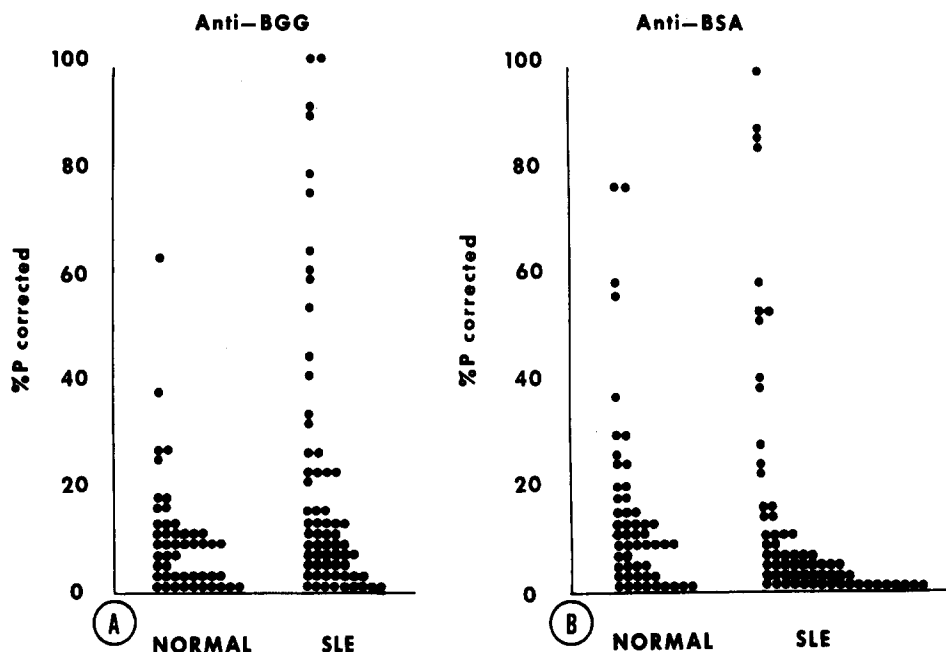


FIG. 3. The per cent of 0.02 μ g N bovine ^{131}I -Fab (A) or ^{131}I -BSA (B) precipitated (per cent P corrected) with normal and SLE sera in the ammonium sulfate test.

In a preliminary experiment, intact ^{131}I -bovine IgG was insoluble in 50 per cent SAS, but less than 10 per cent of the ^{131}I -Fab was precipitated. Therefore, the latter was used as the antigen in antibody studies with the ammonium sulfate test. There were many sera in both normal and SLE groups with significant ^{131}I -Fab binding, but there was again a prevalence of higher binding in SLE sera (Fig. 3, A). Sera from 19 of 47 normal individuals and 33 of 50 SLE patients bound more than 10 per cent of the ^{131}I -Fab. Only 2 normal sera but 14 SLE sera bound more than 30 per cent. In both groups, binding was specifically blocked by BGG and to a lesser extent by sheep gamma globulin.

With ^{131}I -BSA as the antigen (Fig. 3, B), there was also a large number of both SLE and normal sera with significant binding. Twenty-six of 53 normal sera and 21 of 50 SLE sera bound more than 10 per cent. Although more normal sera than SLE sera bound significant amounts of ^{131}I -BSA, there were more SLE sera with high binding. Ten SLE sera but only 5 normal sera bound more than 30 per cent.

The data from the hemagglutination assay and the ammonium sulfate tests were examined with the Kolmogorov-Smirnoff one-tail two-sample test,²⁴ and in both instances the probability that the normal and SLE sera were from one population with respect to BGG antibodies was less than 0.01. However, with respect to BSA antibodies, p was greater than 0.05.

Relationship of anti-BGG in SLE to rheumatoid factor

Sera from 10 patients with rheumatoid arthritis who had rheumatoid factor titers ranging from 1:64 to 1:128,000 were examined for anti-BGG activity

TABLE II. Comparison of rheumatoid factor and anti-BGG hemagglutination titers

Sera	Rheumatoid factor titer	Anti-BGG titer
Rheumatoid arthritis		
1	64	4
2	64	32
3	1,024	<2
4	1,024	4
5	4,096	<2
6	4,096	8
7	4,096	64
8	16,000	16
9	32,000 (<2*)	128 (128*)
10	128,000 (<2)	256 (256)
SLE		
1	<2	32
2	<2	64
3	<2	128
4	<2 (<2)	512 (512)
5	4,096 (<2)	512 (512)

*Titer after absorption with heat-aggregated human Fr II.

(Table II). In addition, the rheumatoid factor titers of 5 SLE sera with anti-BGG were determined. Although 2 of the 10 patients with rheumatoid arthritis had anti-BGG hemagglutination titers greater than 1:64, when these high-titer rheumatoid sera and the one SLE serum with rheumatoid factor were absorbed with heat-aggregated human Fr II until all rheumatoid factor activity was removed (Table II), there was no significant change in their anti-BGG titers.

Antibody reactivity with gamma globulins of various species

Six SLE sera were tested against the gamma globulins of a number of species (bovine, sheep, goat, human, rabbit, pig, dog, rat, and horse) by double immunodiffusion. There was reactivity with bovine IgG and sheep IgG only. Hemagglutination inhibition studies were performed on 6 SLE sera with BGG-coated erythrocytes and bovine, sheep, human, rabbit, pig, and horse IgG's as inhibitors. In all cases isolated bovine IgG was the best inhibitor, although sheep IgG showed some blocking ability. The other species tested showed no significant ability to inhibit.

Three sera were also examined in an ammonium sulfate inhibition system, and again bovine IgG was the best inhibitor, with sheep IgG yielding some inhibition. In contrast to the hemagglutination inhibition studies, in the ammonium sulfate inhibition assay, horse IgG also showed some inhibitory effect with one of the sera tested (Fig. 4).

Immunoglobulin quantitation

Immunoglobulin quantitation was performed on 35 of the reactive sera. Two sera were without detectable IgA. Both had antibovine IgM by immunodiffusion, and one also bound bovine ¹³¹I-Fab. Another serum which was moderately deficient in IgA (38 mg. per cent) had no antibovine IgM but had low binding of ¹³¹I-Fab. All the other sera had normal or elevated levels of IgG, IgA, or IgM.

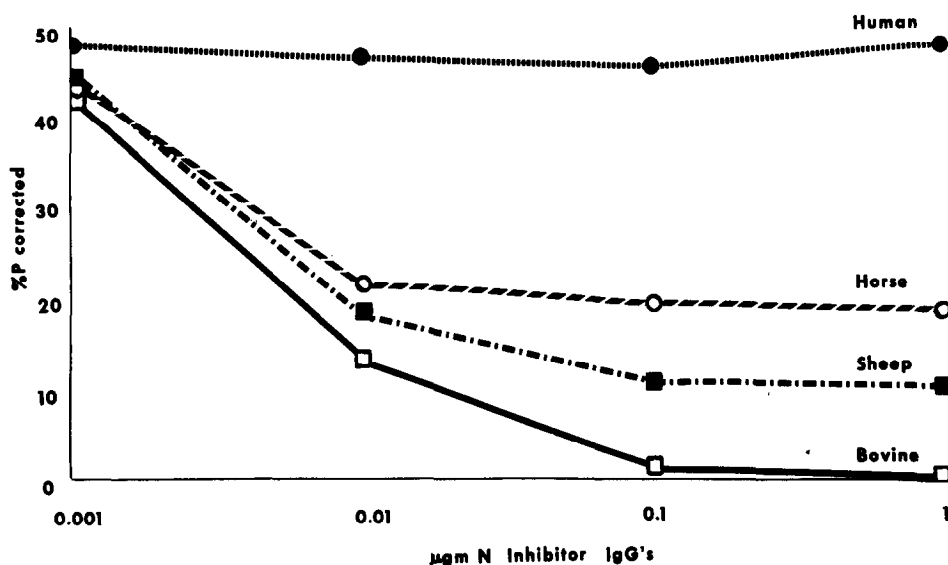


FIG. 4. Ammonium sulfate inhibition curves. Reaction of serum from Patient M. M. with $0.02 \mu\text{g N } ^{131}\text{I}$ -bovine Fab inhibited by gamma globulins from various species. Bovine gamma globulin is the most effective inhibitor, and $1 \mu\text{g N}$ blocks completely. Sheep gamma globulin is a more effective inhibitor than horse gamma globulin, but both show only partial inhibition.

The use of goat antihuman immunoglobulins to quantitate the classes was not a problem²⁵ since none of these sera showed precipitins with normal goat gamma globulin.

BGG in beef extract and milk

All of the sera with precipitins to bovine IgG also reacted with beef extract and milk (Fig. 5), yielding a line of identity with isolated bovine IgG. Extracts from raw and rare beef gave strong precipitin lines, but there was only a faint suggestion of a line with the extract from well-done beef. In addition, skim milk and raw or rare beef were very effective inhibitors in an ammonium sulfate inhibition assay, whereas well-done beef extract was markedly less inhibitory. The milk had an inhibitory effect equivalent to $44 \mu\text{g N}$ bovine IgG per milliliter, raw beef extract contained $11.9 \mu\text{g N}$ IgG equivalents per milliliter, rare beef extract $6 \mu\text{g N}$ IgG equivalents per milliliter and well-done beef extract $0.14 \mu\text{g N}$ IgG equivalents per milliliter.

The occurrence of a BGG-like substance in serum

Seven SLE sera and plasmas inhibited the reaction between rabbit anti-BGG and ^{131}I -bovine Fab by 10 per cent or more. Two plasmas had an inhibitory activity equivalent to more than $0.2 \mu\text{g N}$ BGG per milliliter, enough to allow performance of inhibition titration curves. These data were adjusted as described previously²⁷ and superimposed on the inhibition curve obtained with purified BGG (Fig. 6). Rabbit anti-BGG gave a precipitin line with one of these samples, and the formation of this line was prevented by preabsorption of the rabbit anti-

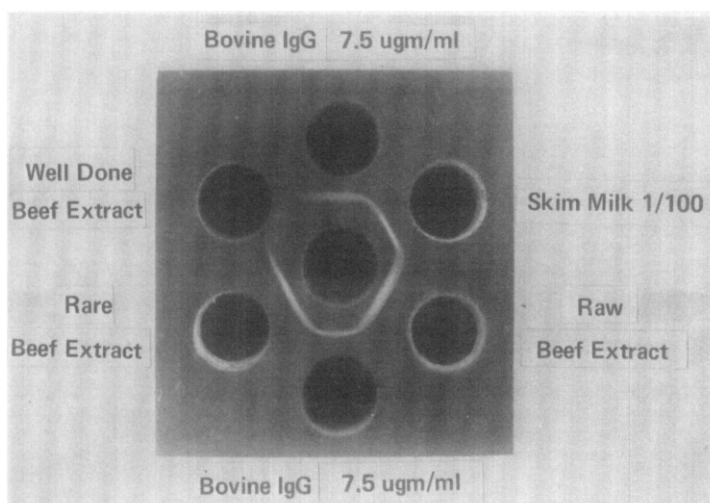


FIG. 5. Reaction of an SLE serum from Patient E. B. in the center well with milk and beef extracts. A line of identity is seen with bovine IgG. Extract of well-done beef shows little, if any, reaction.

serum with BGG. Paradoxically, this SLE plasma bound 35 per cent of $0.02 \mu\text{g N}^{131}\text{I}$ -bovine Fab in an antibody assay. When a sample was fractionated on Sephadex G-200 equilibrated with PBS at neutral pH, the inhibitory activity eluted in the void volume and BGG binding activity eluted in the 7S region (Fig. 7). Thus, the whole plasma appeared to contain both a BGG-like material and anti-BGG activity.

DISCUSSION

Antibodies reactive with a wide variety of endogenous antigens have been found in the sera of SLE patients and intensively studied previously,² but little is known about the possible pathogenic significance of antibodies against dietary antigens to which the patients may be repeatedly exposed. Since such antigens when injected into experimental animals can participate in immune complex disease, a study was undertaken to examine the occurrence of antibodies in human SLE patients to 2 commonly ingested proteins, bovine gamma globulin and bovine serum albumin. Whereas only 3 per cent of a large group of normal sera gave precipitin lines with BGG, 15 per cent of SLE sera reacted. Since this test is relatively insensitive and is capable of detecting only precipitating antibodies, hemagglutination tests were done with red cells coated with intact BGG, and an ammonium sulfate precipitation test was developed with the use of bovine ^{131}I -Fab as test antigen. Both procedures showed that not only is the incidence of antibodies to BGG higher in SLE, but patients with this disease also tend to have higher antibody titers than do the normal subjects with antibody. The use of ^{131}I -Fab as the test antigen in this system poses a special problem because binding would not be detected in sera with antibodies directed only against the Fc portion of bovine immunoglobulins. Therefore, the true incidence of antibodies capable of reacting with bovine gamma globulin may be considerably higher. This

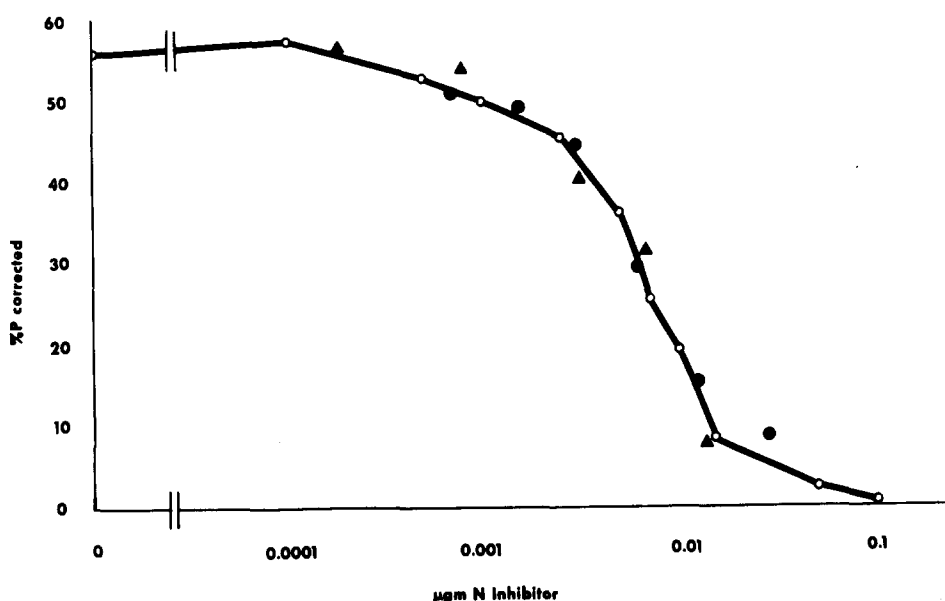


FIG. 6. Inhibition of binding between rabbit anti-BGG and ^{131}I -bovine Fab. Superimposition of 2 SLE and BGG blocking curves when adjusted as previously described.²⁷ ○—○, BGG inhibition curve; ●, estimated BGG in SLE plasma of Patient R. P.; ▲, estimated BGG in SLE plasma of Patient S. S.

is supported by the observation that 2 SLE patients had significant hemagglutination titers against erythrocytes coated with whole BGG and yet had no detectable Fab binding. Currently, we are investigating the use of antihuman gamma globulin as a precipitating agent for BGG-anti-BGG complexes and also the use of the ultracentrifuge, as described previously.²⁸ These techniques are as sensitive as the ammonium sulfate procedure and would allow the use of intact BGG as the test antigen.

Although there was no difference in the over-all incidence of antibodies to BSA, there were twice as many SLE sera with high binding.

The results may be just another example of the high antibody levels to a wide variety of antigens seen in SLE.² However, since both BGG and BSA are commonly ingested proteins, if appropriate amounts of antigenically intact molecules enter the circulation of certain individuals, immune complex disease might occur regardless of the fact that the antibodies are present.

The possibility of a relationship between the anti-BGG antibodies and rheumatoid factor was assessed by examining selected sera of both RA and SLE patients. Although certain sera had both activities present, when they were absorbed with aggregated human Fr II to remove all rheumatoid factor activity there was no detectable change in the anti-BGG titers (Table II). Thus, the hemagglutinins to BGG had a different specificity than did the antibody population responsible for the positive rheumatoid factor tests.

Studies with both the hemagglutination and ammonium sulfate systems showed that the sera were completely inhibited only by bovine IgG. There was evidence of shared antigenicity noted with sheep IgG (Fig. 5), which is not

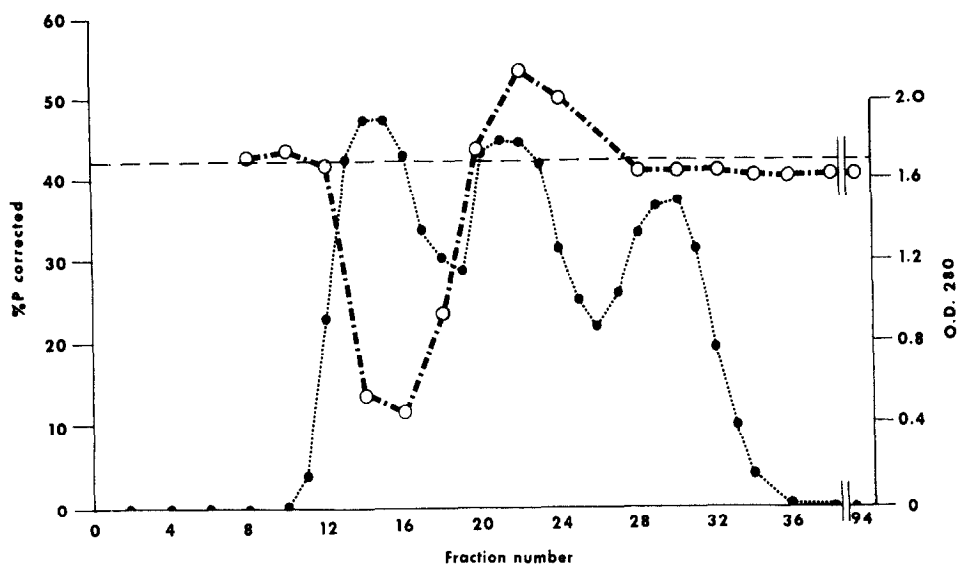


FIG. 7. Inhibition test on G-200 Fractions of SLE plasma of Patient R. P. — — —, Baseline (no inhibition); ● ······ ●, optical density 280 nm. reading on fraction; O — · — O, per cent P corrected in presence of fraction. Between tubes 12 and 17 inhibition occurs, and between tubes 20 and 24 enhancement of binding is seen with the fractions.

surprising considering the close phylogenetic relationship between cattle and sheep.

Quantitative immunoglobulin determinations were performed because a high incidence of antibovine IgM has been found in the sera of IgA-deficient patients.^{25, 26} Thirty-three sera reactive with bovine IgG had normal or increased levels of IgG, IgA, and IgM. There was no correlation between the amount of anti-BGG and the immunoglobulin levels.

The reaction of the sera with beef extracts and milk might be of considerable clinical significance since there is usually daily exposure to such sources of bovine serum proteins. At first it might seem unlikely that intact proteins could cross the blood-gut barrier, but to take part in immune reactions the proteins need only be antigenically reactive and not necessarily intact. Furthermore, 0.001 to 0.064 μ g N BSA per milliliter has been demonstrated in the sera of premature infants fed 0.3 to 1.0 mg. of BSA per milliliter of formula.¹² Although one might argue that the blood-gut barrier is deficient in premature infants, it has also been shown in adult rabbits that after oral intake of BSA, antigenically intact BSA can be detected in the circulation and systemic antibody formation occurs.¹³ Therefore, the possibility that antigenically intact proteins can gain access to the circulation in adult human beings must at least be seriously considered.

A number of SLE sera and plasmas inhibited the reaction between rabbit anti-BGG and ¹³¹I-Fab. When serial dilutions of the 2 most inhibitory samples were tested, blocking curves were obtained which superimposed on that obtained with purified BGG, indicating the inhibitory material was probably BGG or BGG fragments.²⁵ Further evidence in favor of this was the observation that rabbit

anti-BGG gave a precipitin line with one of the plasmas, a line which was prevented by the preabsorption of the rabbit antiserum with BGG. One must be careful to avoid the addition of bovine products while processing human serum or plasma, since it was found that commercial thrombin (of bovine origin), which might be used to clot plasma, contains bovine gamma globulin.

The possibility that BGG and anti-BGG coexist in the sera of some patients with SLE is supported by the results obtained with one SLE plasma. The plasma of Patient R. P. inhibited the binding of ^{131}I -bovine Fab by rabbit anti-BGG when tested in the inhibition system, but paradoxically also bound 35 per cent of $0.02\text{ }\mu\text{g N }^{131}\text{I}$ -bovine Fab in a direct binding assay. After gel filtration at neutral pH, the inhibitory activity of this plasma was in the void volume and the main binding activity in the 7S region. This binding was specifically inhibited by BGG. Thus, this plasma contained both a BGG-like material and antibodies to BGG, supporting the possibility that there might have been immune complexes in the unfractionated plasma. Studies are now in progress to fractionate the other inhibitory sera and plasmas to determine if the inhibitor is in fact BGG or a BGG fragment, and also to determine if there is antibody masked by the inhibitor.

It is of interest that even the highest antibody levels detected represent a relatively low concentration of antibody. Bovine serum had to be markedly diluted to avoid antigen excess with most sera in the immunodiffusion experiments. In addition, rabbits immunized with bovine Fr II in adjuvant had 50 times greater ^{131}I -Fab-binding capacity than the highest titer SLE serum.

Rabbits given daily intravenous injections of BGG or other protein antigens have developed a chronic immune complex glomerulonephritis if the ratio of antibody to antigen in the circulation resulted in the formation and continual circulation of antigen-antibody complexes.⁹ In such cases there was neither marked antibody nor antigen excess, and the complexes formed were too small to be eliminated immunologically yet were large enough to be trapped in the kidney.

An analogous situation might conceivably exist in certain SLE patients; that is, the repeated ingestion of bovine products (milk, meat, etc.) might lead to the recurrent entry of small amounts of immunologically reactive bovine proteins into the circulation. Since the absorbed amounts of antigenically intact protein would probably be small, the pathogenically critical balance of antigen to antibody would most likely occur in sera with relatively low levels of antibody. Most individuals with anti-BGG or anti-BSA do not have evidence of acute immune complex disease, but 2 patients with considerable anti-BGG antibody had smoldering chronic lupus nephritis (1 to 2+ proteinuria, with intermittent hyaline and granular casts, and on renal biopsy the typical granular deposition of gamma globulin and complement along the basement membrane). In addition, several other patients had nephritis but also had circulating DNA antibody, whereas the 2 patients mentioned above had no significant DNA antibody. Patient R. P. on renal biopsy also showed considerable glomerular deposition of gamma globulin and complement but also had DNA antibody.

The proof that BGG-anti-BGG and/or BSA-anti-BSA complexes are involved

in the pathogenesis of lupus nephritis will depend on the demonstration of these complexes in the glomerulus, and this investigation is now in progress. In addition, the effect of removal of bovine and related products from the diet of certain patients with these antibodies and/or circulating antigen will be assessed.

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