

# NATURALLY OCCURRING HUMAN ANTIBODY REACTING WITH BENCE JONES PROTEINS\*,†

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The light (L) polypeptide chains of the human  $\gamma$ -globulins carry antigenic determinants designated as group 1 or 2 (1-3). Allotypic determinants designated InV have been found in association with the L chains of  $\gamma_2$ -globulin (4). Naturally occurring human antibody directed toward L chain determinants has been described in detail only for the InV antigens (5).

The present studies utilize human Bence Jones (BJ) proteins which, in their primary structure, appear to be identical with the L chains of the several human  $\gamma$ -globulins but are entirely of either group 1 or 2 antigenic specificity (1). Tannic acid-treated sheep erythrocytes coated with highly purified group 1 or 2 Bence Jones proteins detect naturally occurring human antibody directed toward L chain determinants which appear to be blocked in intact  $\gamma$ -globulins by the heavy (H) polypeptide chains.

## Materials and Methods

*Bence Jones Proteins*<sup>1</sup>.—Bence Jones proteins were isolated from the urines of patients with multiple myeloma and macroglobulinemia. The isolation of Bence Jones protein 39 from the urine of a patient with Waldenström's macroglobulinemia has been described previously (6). Two urines containing Bence Jones proteins 40 and 43 were dialyzed against water, and then 0.01 M phosphate, pH 8. These BJ proteins were partially purified by stepwise or a salt gradient

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<sup>1</sup> Salts, buffers, and reagents: Reagent grade acetic acid, sodium acetate, and sodium phosphate were used for the preparation of buffers. Mercaptoethanol (California Corp. for Biochemical Research, Los Angeles) and iodoacetamide (Mann Research Laboratories, New York) were used without further purification. Carboxymethyl and diethylaminoethyl cellulose (selectacel 76, 0.8 mEq per gm; and selectacel 70, 0.9 mEq per gm) were products of Carl Schleicher and Schuell Co., Keene, New Hampshire. Sephadex G-75, 100, and 200 were obtained from Pharmacia, Uppsala, Sweden. Twice crystallized pepsin (Lot 673) and crystalline mercuripain (Lot 6102) were products of the Worthington Biochemical Corp., Freehold, New Jersey.

elution from diethylaminoethyl cellulose columns previously equilibrated with 0.01 M phosphate, pH 8. Concentrates of eluted fractions containing Bence Jones protein were separated from any major contaminants of gamma globulin and albumin by gel filtration using a sephadex G-100 column previously equilibrated with 0.1 M NaCl, 0.01 M phosphate, pH 8. Three urinary protein concentrates designated BJ protein 46, 47, and 48, originally obtained as ammonium sulfate precipitates from Dr. E. Osserman, were also passed through similar sephadex G-100 columns under the same conditions. The Bence Jones proteins emerged as the third peak and readily separated from the first peak of 7S  $\gamma$ -globulin and the small second peak of albumin. Other purified Bence Jones proteins were generously supplied by other investigators and were used without further purification. Rabbit antisera to individual BJ proteins of the two major groups and to the  $s_{20,w} = 5(5S)$  fragment of pooled human  $\gamma_2$ -globulin (after pepsin treatment, *vide infra*) were prepared and used to classify all of the BJ proteins by methods described previously (7).

*Isolation of Serum Protein Fractions.*—Pooled normal  $\gamma$ -globulin was Cohn fraction II (FII) Squibb lot 2001.<sup>2</sup> The  $\gamma_2$ -globulin fraction and the macroglobulin-rich euglobulin fraction of individual normal sera were obtained by techniques described previously (8).

*Fragments of  $\gamma_2$ -Globulins.*—

*Pepsin 5S fragment:*  $s_{20,w} = 5S$  fragment was obtained from pooled  $\gamma_2$ -globulin after hydrolysis with pepsin at pH 4.0 for 24 hours by the method of Osterland *et al.* (9). This fragment was examined for residual intact 7S  $\gamma_2$ -globulins by utilizing the Gm a(+) quality of the pooled  $\gamma_2$ -globulin and analyzing the final product for its capacity to inhibit the agglutination of sheep erythrocytes coated with Gm a(+)  $\gamma_2$ -globulin by a serum having anti-Gm (a) specific rheumatoid factor (10).

*Pepsin 3.5S fragment:*  $s_{20,w} = 3.5S$  fragment was obtained from the pepsin 5S fragment by reduction of this fragment with 0.1 M mercaptoethanol for 2 hours in a buffered saline solution at pH 8 and subsequent alkylation with iodoacetamide. Trace-contaminating 5S material was removed by passing the product through a sephadex G-200 column previously equilibrated with a buffered saline, pH 8.

*Papain 3.5S fragment:* The  $s_{20,w} = 3.5S$  fragment termed S by Edelman *et al.* (11) and A by Franklin (12) was formed after proteolysis of pooled  $\gamma$ -globulin with papain and a reducing agent. Residual 7S  $\gamma_2$ -globulin was removed by gel filtration through sephadex G-200 and the A fragment isolated by anionic and cationic cellulose ion exchange chromatography using a system similar to that described by Franklin (12). The absence of contamination of the final product with the F (B) fragment was detected by using a previously described hemagglutination system for the Gm a(+) antigen on this latter fragment (13).

*Fragments of  $\gamma_2$ -Globulin Obtained by Reductive Methods.*—The light (L) polypeptide chains of Cohn fraction II (FII) and of the 3.5S fragment (after pepsin treatment) were obtained by reduction using a variation of procedure 2 described by Edelman and Poulik (14). The reduction was carried out in 0.2 M phosphate, pH 8, rather than in solutions containing urea. Alkylation was with a 5-fold molar excess of iodoacetamide. The L chains of 7S  $\gamma_2$ -globulin were obtained by gel filtration through a G-100 column using 0.5 M propionic acid for elution (15). The L chains (the second peak) were recycled through a sephadex G-100 column to remove contaminating H chains. The L chains obtained from the 3.5S fragment (after pepsin treatment) were separated from the fragments of the H chain by means of a sephadex G-75 column using 1.0 M propionic acid for equilibration and elution (16).

*Storage and Handling of Proteins.*—Many proteins were stored as lyophilized preparations after dialysis against water. In most cases the lyophilized proteins were reconstituted in phos-

<sup>2</sup> Generously provided by the American Red Cross.

phate-buffered saline solution, pH 8. Other protein preparations were simply dialyzed against this buffered saline. All protein solutions were kept frozen prior to use.

*Sera.*—Sera examined for agglutinating substances were obtained from apparently healthy laboratory personnel, patients on the general medical wards, as well as those attending the Arthritis Clinic of this hospital. The agglutinating characteristics of sera from patients with multiple myeloma, macroglobulinemia, and other neoplastic disease states are not included in the present report.

*Serological Tests.*—

*Direct agglutination:* The reference hemagglutination test system used in all studies was the FII hemagglutination procedure utilizing pooled commercial human Cohn fraction II coated on tannic acid-treated sheep erythrocytes by the method of Heller *et al.* (17). Standardization of these cells was carried out, using reference sera from patients with rheumatoid arthritis and all batches of cells showed similar sensitivity within one tube of serial dilution of reference agglutinator. Cells coated with purified Bence Jones proteins or with the L polypeptide chain were prepared in a manner similar to that used for FII cells except that the coating protein was heated in a 1 mg per ml concentration at 55°C for 10 minutes prior to the coating procedure (see Results). Human thyroglobulin was prepared by the method of Spiro (18) and tanned cells coated with this protein were prepared by the method of Boyden (19). Dilution of the agglutinating sera in doubling dilution technique was carried out using a 1:2000 concentration of fetal bovine serum (FBS) in pH 8-buffered saline as the diluent. The agglutination pattern was read after 18 hours at 4°C.

*Inhibition tests:* Serial doubling dilutions of inhibitors were carried out in the 1:2000 FBS diluent prior to the addition of an amount of agglutinator showing two tubes of maximum agglutination in a control test. Inhibitor and agglutinator were maintained at 37°C for 1 hour and then at 4°C for 2 to 4 hours prior to the addition of protein-coated cells. Patterns were read as for direct agglutination.

*Sucrose Density Gradient Studies.*—Continuous linear sucrose gradient from 10 to 40 per cent was prepared as described by Edelman *et al.* (20). Centrifugation of a 1:2 dilution of whole serum was in a Spinco model L machine at 35,000 rpm for 15 hours. Drop samples were collected from a hole in the bottom of the tube. OD 280 was measured in a Beckman DU spectrophotometer.

## RESULTS

The methods used for coating Bence Jones proteins or L chains in general followed those used previously for coating 7S  $\gamma_2$ -globulins when preservation of the Gm specificity during the coating process was essential (21). A protein concentration of 1.0 mg of BJ protein or L chain protein per ml of the buffered saline solution to which tannic acid-treated cells were added appeared to give optimum coating and this concentration was used for all studies in this report. Heating the Bence Jones protein or L chain preparations prior to coating appeared to be a critical matter as regards reactivity with human but not rabbit agglutinators. Table I presents the results obtained when variable durations of heat in a constant bath temperature were applied to the BJ solutions prior to coating. The protein solution was heated in a concentration of 1 mg of protein/ml for the coating procedure. Similar results were obtained using the L chain of pooled normal  $\gamma_2$ -globulins as the cell-coating material. Studies which involved heating of BJ protein and L chains of  $\gamma_2$ -globulin to 70°C for from 5 to

20 minutes yielded no significant increase in activity of the coated cell. Since there appeared no loss of group 1 or 2 specificities by this heating procedure, the application of 55°C of heat for 10 minutes just prior to the coating procedure has become routine in such studies. No increase in agglutinating capacity was found when using the euglobulin fraction in place of whole human serum as an agglutinator with cells coated with BJ proteins or L chains of pooled  $\gamma_2$ -globulin.

Table II demonstrates 16 sera arranged according to ascending FII titers. All have the capacity to agglutinate cells coated with one or another BJ proteins of the two major antigenic groups and/or with the L chains of pooled

TABLE I  
*Effect of Heating Bence Jones Proteins on Ability of Coated Cells to Detect Agglutinators*

Cell coated with	Log <sub>2</sub> titer*		
	Rabbit anti-BJ protein, group 1, No. 40	Rabbit anti-BJ protein, group 2, No. 43	Human serum 4918
BJ protein, group 1, No. 40			
Not heated	7	0	0
Heated 55°C, 5 min.	7	0	3
Heated 55°C, 10 min.	7	0	4
Heated 55°C, 15 min.	7	0	4
BJ protein group 2, No. 43			
Not heated	0	7	1
Heated 55°C, 5 min.	0	7	3
Heated 55°C, 10 min.	0	7	4
Heated 55°C, 15 min.	0	7	4

\* Doubling dilutions of rabbit antisera begin at 1:200 and the human serum at 1:20.

normal  $\gamma_2$ -globulin. It is apparent that some human sera can distinguish between two BJ proteins both of group 1 (*e.g.* No. 7397) and two BJ proteins both of group 2 (*e.g.* No. 6035) by agglutinating cells coated with only one of each pair. The log<sub>2</sub> titer of a serum for BJ protein or L chain-coated cells seems to bear no direct relationship to the log<sub>2</sub> titer of the serum for cells coated with pooled  $\gamma_2$ -globulin. The L chain, derived from the pooled  $\gamma_2$ -globulin, has a different pattern of activity as a cell coating than does the intact  $\gamma_2$ -molecule. There is, however, a trend for sera to show a higher titer for L chain-coated cells the lower the FII titer and for sera to agglutinate L chain coated-cells if they agglutinate cells coated with BJ protein of group 1. An additional expression of this latter relationship will be illustrated subsequently.

Since some agglutinators in human sera appeared capable of reacting with determinants on the L chains of human  $\gamma_2$ -globulins and on naturally occurring

TABLE II  
*Log<sub>2</sub> Titers of Human Sera\* Tested with Various Coated Cells*

Serum	Cell coating					
	Pooled $\gamma_2$ (FII)	L chains of pooled $\gamma_2$	BJ protein, group 1, No. 40	BJ protein, group 1, No. 41	BJ protein, group 2, No. 43	BJ protein, group 2, No. 44
7471	0	3	4	4	1	0
7374	0	3	5	4	0	0
7343	0	5	0	0	0	0
6035	0	0	0	0	4	0
7397	1	4	3	0	0	0
7213	6	5	1	0	0	0
4918	6	3	4	4	5	4
4766	8	1	3	1	2	2
5157	8	0	4	1	4	6
6286	10	0	0	0	5	1
6054	10	0	0	0	2	2
6382	11	0	0	0	6	6
4983	11	1	4	4	3	4
4671	13	2	3	0	0	1
5490	13	0	3	3	3	4
5685	13	0	0	0	3	5

\* First tube contains 1:20 dilution of sera.

TABLE III  
*Inhibiting Capacity of Subunits of  $\gamma_2$ -Globulin and of Bence Jones Proteins for Serum 4918*

Inhibitors*	Number of tubes showing hemagglutination inhibition		
	Cell coating		
	BJ protein, group 1, No. 40	BJ protein, group 2, No. 43	L chain of FII
1. Pooled FII	0	0	0
2. 5S fragment of inhibitor 1 after pepsin-acid treatment	0	0	0
3. 3.5S fragment from reduction and alkylation of inhibitor 2	0	0	0
4. 3.5S (A-C) fragment after papain treatment of inhibitor 1	0	0	0
5. L chain after acid dissociation of inhibitor 3	4	0	8
6. BJ protein, group 1, No. 40	4	0	2
7. BJ protein, group 2, No. 43	0	4	0

\* All inhibitors were in an initial concentration of 0.1 mg of protein/ml.

L chains in the form of BJ proteins, while having no agglutinating activity toward intact  $\gamma_2$ -globulin, it seemed possible that the involved L chain determinants were inaccessible in the intact molecule.

Serum 4918 used in the study outlined by Table III is from a young woman with acute pyelonephritis. This serum demonstrates agglutinating capacity for several available BJ proteins of group 1, group 2, and L chains of pooled  $\gamma_2$ -globulin (FII). Concentrations of the serum which provide equal agglutinating activity for the three cell coatings shown were added to 2-fold dilutions of a series of potential inhibitors. All inhibitors were of the same protein concentration.

The failure of intact  $\gamma_2$ -globulin to affect inhibition has been confirmed at OD 280 = 1.0 using intact  $\gamma_2$ -globulin from ten normal individuals. A single  $\gamma_{1M}$ -preparation of antigenic group 1 from a patient with macroglobulinemia was equally ineffective as an inhibitor. Proteolysis of the F portion of the H chains of  $\gamma_2$ -globulin by pepsin at pH 4.0 (inhibitor 2) failed to reveal the determinant sites on the L chains. Reduction and alkylation of the 5S fragment produced by pepsin-acid treatment yielded an  $s_{20,w} = 3.5$  fragment (inhibitor 3) and neither this nor a similar product obtained directly from  $\gamma_2$ -globulin by papain proteolysis (inhibitor 4) demonstrated any inhibitory capacity. It was only by acid dissociation of L chain from the fragment of H chain contained in the  $s_{20,w} = 3.5$  material that a material (inhibitor 5) was obtained which proved capable of competing with the L chain or BJ protein of group 1 coated on the cells for the agglutinators in serum 4918. L chain obtained directly from  $\gamma_2$ -globulin behaved in a manner indistinguishable from that of inhibitor 5. The BJ proteins which were capable of the specific inhibition shown in Table III had, of course, not been subject to the several procedures required to obtain inhibitors 2 to 5. The ability of individual BJ proteins of group 1 to inhibit hemagglutination which involved cells coated with the L chain of pooled  $\gamma_2$ -globulins appeared to be quite variable and depended in part on the particular human serum used as the source of agglutinator. None of 14 BJ proteins of group 2 tested have demonstrated the capacity to inhibit hemagglutination of cells coated with L chains of normal pooled  $\gamma_2$ -globulins. Neither have these 14 BJ proteins of group 2 inhibited hemagglutination of cells coated with any of three BJ proteins of group 1. These two specificities have been equally clear in the reverse situation.

As shown in Table II selected sera were capable, by their direct hemagglutinating specificity, of distinguishing between two BJ proteins belonging to the same group as established by rabbit antisera to individual BJ proteins. In Tables IV *a* and IV *b* are shown subgroupings of groups 1 and 2 as  $\log_2$  titers expressing the inhibitory capacity of BJ proteins. In Table IV *a* are six group 2 BJ proteins tested against two different agglutinators of cells coated with BJ protein group 2, No. 46. The grouping of inhibitors and non-inhibitors is clearly different when different sera, both from normal individuals, serve as the source

of the agglutinators. Sera which can affect such subdivision of the major groups have to date been more easily found when studying group 2 rather than group 1 BJ proteins. An example of subgroupings of BJ proteins of group 1 by their inhibitory behavior is shown in Table IV *b* and again this delineation depends on the agglutinators and, in this case, the cell-coating material.

TABLE IV *a*  
*Log<sub>2</sub> Titer of Inhibitors for Group 2-Specific Systems*

Inhibitors (initial OD 280 = 0.1)	Cell coating	
	BJ protein, group 2, No. 46	BJ protein, group 2, No. 46
	Agglutinator serum	
	5491	6042
BJ protein, group 2		
No. 43	6	0
No. 45	0	0
No. 46	7	5
No. 47	0	4
No. 48	0	0
No. 49	0	4

TABLE IV *b*  
*Log<sub>2</sub> Titer of Inhibitors for Group 1-Specific Systems*

Inhibitors (initial OD 280 = 0.2)	Cell coating	
	BJ protein, group 1, No. 40	BJ protein, group 1, No. 41
	Agglutinator serum	
	4918	4893
BJ protein, group 1		
No. 39	0	3
No. 40	5	0
No. 41	6	5

*Nature of the Agglutinating Substances in Human Sera.*—Of some 100 sera found to demonstrate agglutinating specificity directed toward cells coated with the L chains of normal pooled  $\gamma_2$ -globulins or BJ proteins of the two major groups, only one serum has had an hemagglutination titer greater than 1:1000. Twenty  $\gamma_2$ -globulin preparations from these sera obtained by anionic exchange chromatography have failed to demonstrate this hemagglutinating capacity.

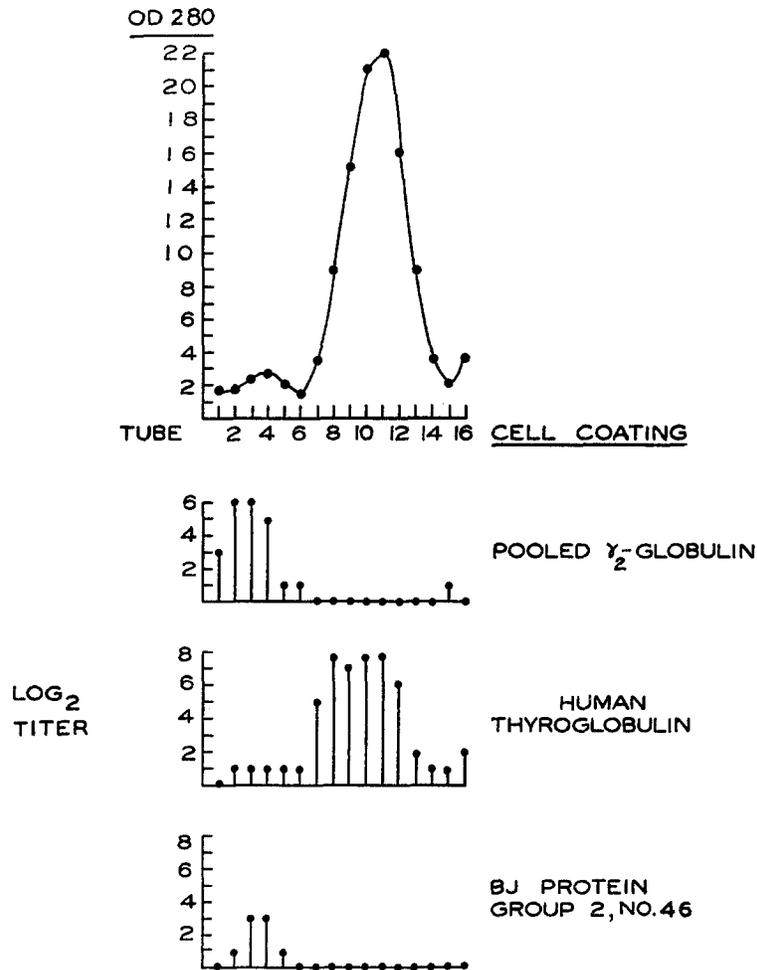


FIG. 1. Fractionation of serum 7501 by density gradient ultracentrifugation. Log<sub>2</sub> titers of each tube fraction for three different coated cells.

Agglutinating activity of the whole serum is markedly reduced or abolished by treatment with 0.1 M mercaptoethanol for 8 hours. The macroglobulin-rich fraction obtained by gel filtration of serum in low ionic strength buffer does reveal the agglutinating activity of the whole serum in titers comparable to that of the whole serum. Fig. 1 demonstrates that on sucrose density ultracentrifugation of a serum which demonstrated rheumatoid factor, thyroglobulin antibody, and BJ protein group 2 antibody activity, the agglutinators for BJ protein-coated cells have the same distribution as do the rheumatoid factors. These results appear to strongly suggest that the agglutinating factors for BJ

protein and the L polypeptide chains found in human sera are macroglobulin  $\gamma$ -globulins.

#### DISCUSSION

The experiments just described demonstrate that human sera may contain an agglutinating substance or substances whose specificity is directed toward determinants found on human Bence Jones protein of both major antigenic groups. Williams (22) has reported weak agglutinating ability of some 19S rheumatoid factors for cells coated with the L chain of human or rabbit  $\gamma$ -globulin, and in the present study this has been confirmed using the isolated L chains of pooled normal human  $\gamma_2$ -globulins. Since the agglutinating substances appear to be contained within the macroglobulins and since their specificity is directed toward a naturally occurring constituent of human  $\gamma$ -globulins, past arguments for human rheumatoid factors being antibody (iso- and/or auto-) are applicable to these agglutinators. These arguments have recently been presented in detail (23).

The necessity for heating the protein just prior to coating on cells suggests similar observations made when coating  $\gamma_2$ -globulins of known Gm type (10, 21). In both instances rabbit 7S antibody to the material used for cell coating reacted with cells coated with unheated protein, while only cells coated with previously heated protein were agglutinated by agglutinators found in human sera. It appears likely that both the application of heat and the actual cell-coating procedure result in conformational changes of the coating protein which allow interaction with macroglobulin antibody. Whatever the nature of the change produced by heat, it is interesting that such a change had not occurred during the several relatively drastic chemical steps required in the preparation of the L polypeptide chains of human  $\gamma_2$ -globulins or if the change had occurred it was reversible. That conformational changes in Bence Jones proteins occur at the temperature used in this study recently has been demonstrated by Gally and Edelman (24) in their measurements of the fluorescence of heated BJ proteins. The essential role of what are probably conformational factors on the serological activity of globulin-coated cells has been demonstrated by Pirofsky *et al.* (25). In prior studies of Gm-specific  $\gamma_2$ -globulins coated on tanned cells, it was found that the euglobulin fraction of some sera could cause hemagglutination when the whole serum could not (21) thus suggesting an autoinhibitory phenomenon. Similar attempts to use the euglobulin fraction of sera which fail to or weakly agglutinate cells coated with BJ or L chain protein failed to indicate autoinhibition for this system. Although limited in number, the sera presented in Table II illustrate that human agglutinators for BJ proteins are distinct from antibody to H chain determinants. All attempts to prove that coating  $\gamma_2$ -globulin on cells exposes the L chain sites toward which human antibody appears directed have failed. In no instance have L chains or any examined BJ protein been capable of inhibiting the hemagglutination reaction

between  $\gamma_2$ -globulin-coated cells and naturally occurring human agglutinators. Table II is meant only to illustrate the problem of subgroup classification as it is emerging since it is not possible to assemble a panel of BJ proteins of the two major groups which we could be certain would detect agglutinators for all possible subgroups. For this same reason, nothing in this report is meant to imply knowledge of the distribution of BJ protein antibody in human sera by age, family relationship, or clinical state.

That the specificity of these agglutinators conforms to the two major BJ protein groups is best illustrated in the failure, to date, of a BJ protein of one group to inhibit a test system in which a human serum causes agglutination of cells coated with a BJ protein of the other group (Table III). The use of naturally occurring human antibody would appear to extend the complexity of the BJ protein subgroup classification beyond that suggested by rabbit antisera using precipitation techniques (26). From Table IV *a* it would appear that serum 5491 has agglutinating specificity for a determinant of BJ group 2 protein No. 46, different from that of serum 6042. It is by the extension of this technique that one might elaborate the determinant mosaic within each major antigenic group. That this subgrouping may be more complex for group 2 than group 1 is suggested by the work of Putnam *et al.* (27) using peptide maps of BJ proteins of the two major antigenic groups, by Stein *et al.* (26) using double diffusion studies in agar with rabbit antisera to Bence Jones proteins, and in the present study by the difficulty in finding agglutinators which distinguish among BJ proteins of group 1 compared to the ease in finding discriminating sera for group 2. These results are also explainable, however, by the possibility that the particular BJ proteins of group 1 currently available share many determinants of the same subgroup. Failure to observe cross-reactivity between any Bence Jones protein of group 2 and L chain of pooled  $\gamma_2$ -globulin while such cross-reactivity is observed with BJ proteins of group 1 and L chains suggests the greater individuality of members of group 2 and therefore the probability that there would not be a sufficient concentration of a given subgroup in the L chains of pooled  $\gamma_2$ -globulins to compete with the homogeneous population of a BJ group 2 protein.

The presence of human antibody to configurations of human  $\gamma_2$ -globulin which are blocked in the intact molecule has been demonstrated previously using the "5S" fragment obtained by the pepsin-acid treatment of human  $\gamma_2$ -globulin (9, 28). Cells coated with such fragments are agglutinated by select human sera and this hemagglutination is not inhibited by intact  $\gamma_2$ -globulin or isolated L chains. The results shown in Table III indicate that it is the portion of the H chains which survives proteolysis using pepsin that furnishes the steric obstruction to the L chain determinants. After reduction of the 5S fragment, the H portion of the fragment is bound to the L chain by non-covalent forces

and is still capable of the obstruction of the determinants toward which this antibody is directed.

Although the explanation just presented makes possible the concept of the simultaneous circulation of intact  $\gamma$ -globulins and antibodies to obstructed L chain sites, it does not deal with what have been described as free L chains present in small amounts in normal sera (29). The source and time of antigenic stimulus for the antibody system just described is, as yet, unknown. Preliminary observations of human umbilical cord blood indicate detectable levels of these macroglobulin antibodies present at birth. This fact, together with a specificity of such antibody directed toward determinant groups present on all classes of immune globulins, suggests the possibility of an homeostatic system. The biological significance of human antibody to exposed determinants of human  $\gamma$ -globulins such as the Gm and InV sites as well as the significance of antibody to buried or obstructed determinants such as those just described remain a problem for continued investigations.

#### SUMMARY

Agglutinating substances having characteristics of naturally occurring macroglobulin antibodies to human Bence Jones proteins have been identified in human sera. By means of hemagglutination and hemagglutination inhibition techniques, common determinants have been demonstrated on the light (L) polypeptide chains of pooled normal human  $\gamma_2$ -globulin and on some Bence Jones proteins of group 1 but not of group 2. Individual human sera serve to delineate subgroups of the two major antigenic groups of the Bence Jones proteins by agglutinating cells coated by one but not another protein of the same antigenic group. The complexity of subgroups, especially of group 2, is established by testing a panel of Bence Jones proteins of the same group for their ability to inhibit hemagglutination. By this means it appeared that different sera recognized different group-specific determinants of cells coated with a single Bence Jones protein.

The capacity of the L polypeptide chains and proteolytic fragments of  $\gamma_2$ -globulin to inhibit the hemagglutination reaction between Bence Jones protein or L chain-coated cells and human sera was examined. These studies demonstrated that the determinants, toward which agglutinators of human serum are directed, appear to be blocked in intact  $\gamma_2$ -globulin and in all fragments in which H chain remains in proximity to L chain. It would appear that the presence of H chains bound to L chains by non-covalent bonds completely obstructs the reactivity of the involved L chain groups. The agglutinating capacity of a serum toward Bence Jones proteins or L chains of  $\gamma_2$ -globulin appeared to be independent of its agglutinating capacity for cells coated with intact  $\gamma_2$ -globulin. No correlation of the presence in serum of agglutinators for Bence Jones proteins or L chains with health or disease has been established.

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*Note Added in Proof.*—Since submission of this manuscript, R. C. Williams, Jr., (*Proc. Nat. Acad. Sc.*, 1964, **52**, 60) has reported on agglutinators in the sera of persons with rheumatoid arthritis which react with Bence Jones protein coated cells. His report finds no correlation of agglutinating activity with the InV (a) type of the Bence Jones proteins.

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