Some Immunochemical Relations of Bence-Jones and Hyperglobulinemic Serum Proteins of Multiple Myeloma Patients*

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The origin of Bence-Jones proteins and their relationships to normal serum proteins and to the globulins often seen in large amounts in the sera of multiple myeloma patients have been the subject of numerous investigations (2, 6, 9, 13, 14, 16, 19). Putnam and Hardy (14) indicated, on the basis of isotopic tracer studies, that these hyperglobulinemic serum proteins do not break down to form Bence-Jones proteins, nor do the latter appear to be the sole immediate precursors of the myeloma serum globulins.

Bence-Jones proteins have been found either to possess a very low level of or to lack methionine (1, 3, 17, 18). It has been suggested that this might represent a defect in methionine incorporation into certain protein structures and result in a limited capacity to incorporate such fragments into a serum globulin that was undergoing an accelerated synthesis (6). These incomplete globulin fragments would be the Bence-Jones proteins.

Such proteins as a class are physicochemically heterogeneous (5, 15, 16) and also differ immunologically, although they are related antigenically to normal serum globulins (6, 9, 19). If the Bence-Jones proteins are incomplete synthetic products of certain hyperglobulinemic serum proteins, it would be expected that they would show stronger immunological relationships to such proteins from the same patient source than to normal globulin or to the elevated serum globulins of other myeloma patients. The present work indicates that such relationships are indeed strongest within a given patient. This type of approach had been made previously by Moore, Kabat, and Gutman (11), but the type of proteins used and an oversimplification of the problem did not permit a definite answer to this question.

MATERIALS AND METHODS

The various Bence-Jones proteins (BJ) utilized were purified by a combination of salt and ethanol fractionation technics. The γ_2-globulins of normal human serum (γ_2-Nor.) and the various myeloma serum globulins were separated by ethanol fractionation procedures. The myeloma proteins will be referred to as MM, and the prefix letters to these and the BJ proteins will designate the patient source.

Two multiple myeloma patients, SI and RA, had both elevated serum globulins and urinary BJ proteins of suitable physical properties. Other myeloma patients were used to provide either hyperglobulinemic serum proteins or BJ proteins. Within the limits of electrophoretic and ultracentrifugal methods of assay, the MM and BJ proteins showed no cross-contamination.

Rabbits were immunized to the various proteins studied by the adjuvant technic of Freund and McDermott (8). The rabbit γ-globulins were then separated by the method of Nichol and Deutsch (12). These were reconstituted into an isotonic saline-borate buffer of pH 7.4 in an amount sufficient to give a maximum specific precipitate of 100–250 μg. N/ml of antibody solution. The quantitative precipitin reactions were carried out in a 4- or 6-ml. volume. The reactants were first held at 37° C. for 1 hour and then placed in the cold (1°–2° C.) for 2–3 days. The specific precipitates were removed by centrifuging near 1° C., washed 3 times with 0.15 M NaCl at 0° C., and the amount of nitrogen then determined by a micro-Kjeldahl method.

Experiments designed to show combination of all the antibody to a given protein by a heterologous antigen were carried out on amounts of antibody preparation giving maximum specific precipitates of 200–250 μg. N. Sufficient amounts of the heterologous combining protein were added to put the systems into the completely soluble antigen excess zone. These reaction mixtures were then tested for residual antibody activity by adding from 2 to 4 μg. of homologous antigen N. Blocking or inhibition reactions were carried out in the manner previously described (6, 7)

RESULTS AND DISCUSSION

Charts 1 and 2 show results of the precipitin reactions of various serum globulins and BJ proteins with antibodies to SI-BJ and RA-BJ, respectively. In both cases the strongest cross-reaction occurred with the homologous MM protein. In addition to the systems charted, two other BJ proteins (ZE and LP) and three other MM proteins (KL, MI, and CA[1]) were employed as antigens in these systems. The isoelectric points and sedimentation constants of all fractions studied immunologically are shown in Table 1. The MM systems KL, MI, and CA also cross-reacted with the antibodies to SI-BJ and RA-BJ

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[1] CA-MM are the macroglobulinemic proteins with the properties previously reported (10).

but more weakly than did the γ_2-Nor. LP-BJ failed to show any specific precipitate with either antibody preparation over antigen additions from 8–200 μg. N. ZE-BJ similarly failed to react with anti-SI-BJ and reacted only weakly with anti-RA-BJ. The extent of the cross-reactions of the various proteins with the SI-BJ and RA-BJ antibodies are presented in Table 2.

Failure to give a specific precipitate does not necessarily demonstrate the absence of an immunochemical reaction. The reaction of the ZE-BJ proteins with the antibodies in question can be demonstrated by inhibition tests based on their ability to partially block the homologous reaction.

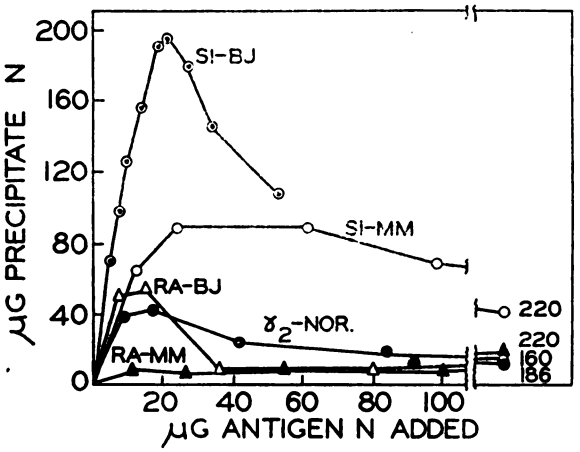

CHART 1.—Quantitative precipitin reactions of various proteins with rabbit antibody to the SI Bence-Jones proteins.

Their blocking ability is demonstrated by the data of Table 3. Thus ZE-BJ, which did not cross-react to an appreciable extent, showed very strong blocking activity. A similar behavior of KL-MM with ZE-BJ and LP-BJ antibodies has been noted (6).

The ability of MM proteins KL and CA to react weakly in cross-reactions with SI-BJ and RA-BJ antibodies but to show no blocking reaction suggests that their cross-reactions are due to antibody to impurities present in small amounts in these BJ proteins.

A significant blocking of the BJ antibodies by γ_2-Nor. at the level tested is indicated by the data of Table 3. When 112 mg. of γ_2-Nor. was added to amounts of RA-BJ and SI-BJ antibodies giving specific precipitates of 200–250 μg. N, all the BJ antibodies were combined. This indicates that all the antigenic determinants of these two BJ proteins are contained in the normal serum γ-globulins employed. Two control systems, one absorbed with 182 mg. of a crude bovine γ-globulin preparation (4) and the other with 138 mg. of normal human serum albumin, did not remove the antibody activity.

Other immunological cross-reactions employed antibody to SI-MM, RA-MM, and γ_2-Nor. The reactions of RA-BJ and SI-BJ with their corresponding MM antibodies were extremely weak in comparison with the reverse immunological reaction. Charts 3 and 4 illustrate reaction results

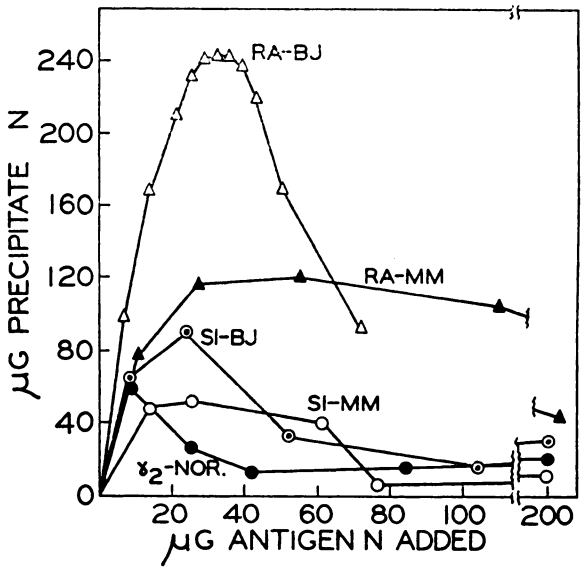

CHART 2.—Quantitative precipitin reactions of various proteins with rabbit antibody to the RA Bence-Jones proteins.

TABLE 1

ISOELECTRIC POINTS AND SEDIMENTATION CONSTANTS OF PROTEINS USED

Protein system*	Isoelectric point	Sedimentation constant (S)†
ZE-BJ (6)		‡
LP-BJ (5)	5.3	3.5
SI-BJ	5.52	3.26
RA-BJ	6.15	3.48
SI-MM	6.25	6.65
RA-MM	7.4	6.54
KL-MM (7)	7.4	6.10
MI-MM (7)	8.1	6.39
CA-MM (10)	6.33	18.0, 21.2, 26.4
γ_2-Nor. (7)	7.25	6.43

* Reference numbers following the proteins are source of the physical and other data for these proteins.

† $s_{20,w} \times 10^{-13}$ cm. sec.$^{-1}$, extrapolated to zero concentration.

‡ Contained several ultracentrifugal entities.

obtained with SI-MM and γ_2-Nor. antibody. The reaction of γ_2-Nor. antibody was stronger than that of SI-BJ. It has been previously demonstrated that normal serum globulins can completely combine antibody to the MM proteins (7). However, if the BJ proteins are only a piece of the MM proteins, they should not be able to effect this.

At a high level of antigen (10 mg. of protein N), SI-BJ was in complete antigen excess, and all the antibody to SI-MM had been combined. Because of its low molecular weight, SI-BJ would be expected to give a more sharply rising precipitin reaction (i.e., curve) and a more rapid attainment of a soluble antigen excess zone than proteins of the molecular weight of MM or γ_2-Nor. However, it can be seen from Charts 3 and 4 that such was not the case. This result, and the relatively small amounts of specific precipitate formed on the addition of SI-BJ, suggest that, in addition to a strong blocking reaction, there also must be small amounts of impurities of the γ_2-Nor. type in the BJ protein. This also obtains for the RA-MM system. An amount of this antibody giving from 200 to 250 μg. of specific precipitate N in the homologous reaction could be completely combined by 138 mg. of RA-BJ protein. This type of result appears to be due to reactions complicated by immunological cross and blocking reactions and the presence of impurities and must, therefore, be interpreted in terms of the amounts of reactants and the stoichiometry of the systems involved. The reactions of SI-MM and SI-BJ with γ_2-Nor. antibody (see Chart 4) are likewise illustrative of the above considerations. Korngold and Lipari (9) have reported such type reactions of BJ and MM proteins with antisera to γ-globulin. The possibility always obtains that the MM or BJ proteins contain small amounts of γ_2-Nor. proteins or proteins capable of cross-reacting strongly with its antisera. This would tend to give increasing amounts of specific precipitate on large addition of antigens, as noted for Chart 4.

The experimental results presented indicate that antisera to BJ proteins cross-react strongly

TABLE 2

CROSS-REACTIONS OF VARIOUS MYELOMA GLOBULINS AND BENCE-JONES PROTEINS WITH SI-BJ AND RA-BJ ANTIBODIES

	SI-BJ ANTIBODY		RA-BJ ANTIBODY	
ANTIGENS	Max. ppt. (μg. N)	Per cent cross-reaction*	Max. ppt. (μg. N)	Per cent cross-reaction*
SI-BJ	197	100	90	36
SI-MM	95	48	51	20
RA-BJ	55	28	250	100
γ_2-Nor	44	22	54	22
KL-MM	25	13	45	18
CA-MM	16	8	12	5
MI-MM	16	8	25	10
RA-MM	10	5	121	48
ZE-BJ	0	0	9	4
LP-BJ	0	0	0	0

* The per cent cross-reaction is defined as the maximum specific precipitate given by the heterologous antigen $\times 100$ divided by the maximum specific precipitate given by the homologous antigen.

TABLE 3

IMMUNOCHEMICAL BLOCKING OF SI-BJ AND RA-BJ ANTIBODIES BY VARIOUS PROTEINS*

BLOCKING PROTEIN	μg. N USED	PER CENT HOMOLOGOUS REACTION BLOCKED† Anti-SI-BJ	Anti-RA-BJ
SI-BJ	89		92
SI-MM	126	80	55
RA-BJ	90	95	
RA-MM	121	51	100
γ_2-Nor.	93	22	24
KL-MM	85	0	7
CA-MM	130	0	6
LP-BJ	119	0	0
ZE-BJ	117	81	89

* The homologous antigen-antibody reaction was set up in slight antigen excess to make it sensitive to antibody combination by heterologous antigen.

† Per cent of homologous specific precipitation blocked by addition of heterologous antigen is referred to here as being equivalent to:

$$\frac{(\text{homologous ppt.} + \text{heterologous ppt.}) - \text{heterologous ppt. alone}}{\text{homologous ppt.}} \times 100 .$$

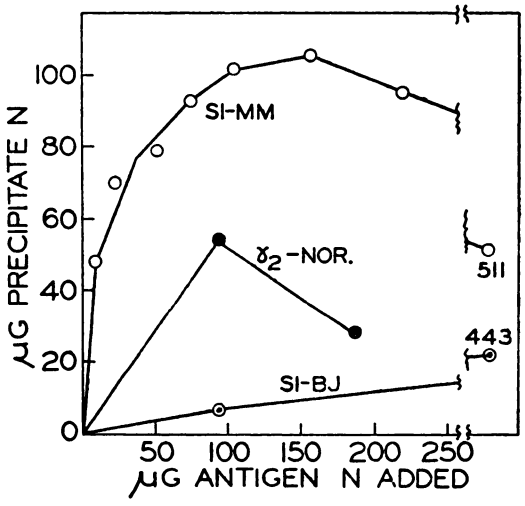

CHART 3.—Quantitative precipitin reactions of various proteins with rabbit antibody to the SI elevated multiple myeloma serum globulin.

with their corresponding MM proteins but that the reverse is not true. An explanation for this may be offered in terms of our suggestion that the BJ proteins may represent incomplete synthetic steps in the production of MM proteins. It is well recognized that most BJ proteins are relatively poor antigens. When immunizing with a serum globulin isolated from a case of multiple myeloma, the antigenic determinants corresponding to the BJ portion might be expected to call forth little antibody production relative to other determinants in the MM molecule. Hence, BJ proteins should react weakly with antibody to MM protein. Immunization with the BJ protein forces the production of antibody toward what

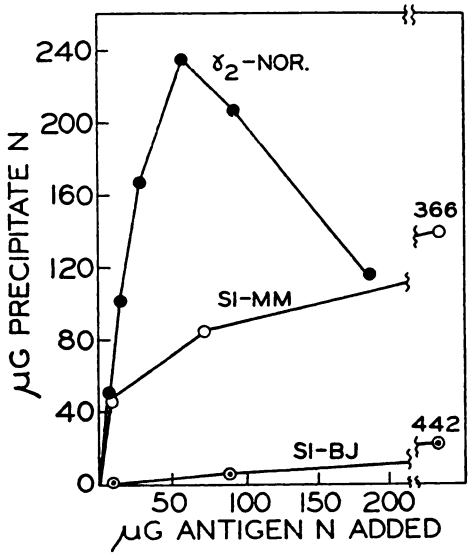

CHART 4.—Quantitative precipitin reactions of various proteins with rabbit antibody to normal human serum γ_2-globulin.

may be relatively weak antigenic determinants in the MM protein, since determinants characteristic of other parts of the MM protein are absent. Since the MM protein contains the BJ antigenic determinants, it should cross-react strongly with the BJ antisera. Various previous experimental data do not obviate this concept, and additional evidence is presented here. Considerations related to the nature of the antigenic determinants in MM, BJ, and γ_2-Nor. proteins such as those suggested above have also been proposed by Korngold and Lipari (9) to explain immunological reactions encountered in working with these systems.

SUMMARY

Bence-Jones proteins separated from the urine of multiple myeloma patients showed greater immunochemical similarities to the hyperglobulinemic serum proteins of the same patient than to those of unrelated ones or to normal γ-globulins.

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