Sharing of Individual Antigenic Determinants Between a γG and a γM Protein in the Same Myeloma Serum[1] (35116)

G. M. PENN,[2] H. G. KUNKEL, AND H. M. GREY

The Rockefeller University, New York, New York 10021; and Scripps Clinic and Research Foundation, La Jolla, California 92037

The predominant variable region antigens of the immunoglobulins are the individual antigenic or idiotypic determinants. These unique determinants are specific for individual myeloma proteins or isolated antibodies and they are not detected in the proteins of other sera (1, 2). They are found on both isolated heavy and light chains and also relate to the conformational structure of the heavy and light chain combination (2) and the polymer conformation of γM proteins (3). The amino acid sequence location of these antigens and their genetic control especially in relation to the constant regions of the heavy chain are essentially unknown.

In addition to the individual determinants, other antigens subdivide the heavy and light chains into variable region subclasses. In the case of the light chains the variable region determinants group both kappa and lambda light chains into three or more subclasses with each number of the subclass having markedly similar N-terminal amino acid sequences (4). These antigens, however, are not shared between kappa and lambda light chains. In contrast, the heavy chains contain variable region determinants which are shared between heavy chain classes (5). In the rabbit some of these determinants appear to serve as genetic markers and have been correlated with the amino acid sequence of the N-terminal cyanogen bromide cleaved fragment of the heavy chain (6, 7).

The present report deals with the sharing of individual antigenic determinants by a γM and a γG3 protein in the same myeloma serum. Different antisera demonstrated that shared individual determinants were present in the heavy chains of these two proteins. Antisera made against isolated light chains also showed identical individual specificity for the light chains of the two proteins.

Materials and Methods. Source of Material. Plasma was obtained by plasmapheresis from a 67-year-old Caucasian male (Mi) with clinical features of malignant disease and a retroperitoneal plasmacytoma. Immunoelectrophoresis of the patient's serum demonstrated two homogenous proteins (γM lambda and γG3 lambda). The γM and γG3 were isolated by combined pevikon block electrophoresis and gel filtration on a 5 *M* agarose column equilibrated with phosphate buffered saline, pH 7.3.

Isolation of heavy and light chains and enzymatic cleavage of γG3. The γM and γG3 were reduced as previously described (8). Twenty mg of protein in 0.55 *M* Tris, pH 8.2, was reduced for 1 hr at room temperature with 0.1 *M* 2-mercaptoethanol. Alkylation with 0.11 *M* iodoacetamide proceeded in the cold for 1 hr and was followed by overnight dialysis against cold 1 *M* propionic acid. The light and heavy chains were separated on a Sephadex G-200 column equilibrated with 1 *M* propionic acid at 4°. This procedure produced three protein peaks. The first peak contained heavy chains and contaminating light chains, while the second and third peaks contained heavy and light chains, respectively. Only the second and third peaks were utilized throughout the course of this investigation. γM_s was produced by reduction and alkylation as described above, followed by dialysis against 0.15 *M* phosphate buffered saline.

$F(ab')_2$ fragments of the γG3 were ob-

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tained by digesting the isolated γG3 with 2% (w/w) pepsin (Worthington) in 0.1 *M* acetate buffer, pH 4.0, at 37° for 16 hr (9). The products of digestion were subsequently dialyzed against 0.15 *M* phosphate buffered saline, pH 7.3, and used for immunization without further purification.

Preparation of antisera. Three types of antisera were prepared by the immunization of rabbits. Three antisera (type I) were prepared by injecting the light chains derived from Mi γM. Four antisera (type II) were made by immunization with the pevikon block isolated mixture of Mi γM and Mi γG. Two antisera (type III) were obtained by utilizing the Mi γG3 $F(ab')_2$ fragment as the antigen. The antisera were absorbed with pooled isolated kappa and lambda light chains; normal human serum; and myeloma proteins of the same class, subgroup, and allotype in order to obtain individual antigenic specificity.

Precipitin analysis and hemagglutination inhibition. Ouchterlony analyses were performed by standard techniques using 0.6% agarose (Difco Laboratories, Inc., Detroit) in barbital buffer (pH 8.5, 0.045 *M*).

Hemagglutination inhibition was performed by the bis-diazotized benzidine (BDB) procedure as previously described (10). One vol of packed O, human red cells was added to 1 vol of a stock BDB solution diluted 1:15 in phosphate buffered saline (0.15 *M*) at pH 7.3. The mixture was left for 2 min, and 10 vol of protein at 1–2 mg/ml were added. After 15 min, the coated cells were washed and used for typing.

Results. Light chains. The myeloma serum (Mi) was shown to contain a γM and a γG3 protein, both proteins having similar mobilities by pevikon block electrophoresis. However, they were readily separated by subsequent 5 *M* agarose column purification. Each protein was demonstrated to have only lambda light chains. Antisera type I were prepared in rabbits against the light chains of the γM protein and were utilized as demonstrated in Fig. 1. In this Ouchterlony plate analysis both unabsorbed and absorbed individually specific antisera are used to compare the antigenic determinants between the two light chain preparations and heterologous kappa and lambda Bence Jones proteins. The resultant precipitin reaction with the unabsorbed antiserum shows that the two lambda chains contain identical antigenic determinants and that they show only partial identity with the heterologous lambda light chains. These precipitin differences are still apparent even after absorption of the antiserum with normal human serum and pooled lambda Bence Jones proteins. In this system the light chains of the γM and γG appeared to contain identical individually specific determinants.

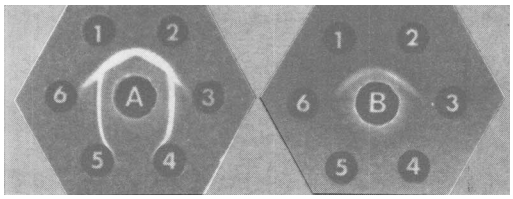

FIG. 1. Immunodiffusion precipitin reactions with rabbit anti-light chains from Mi γM: (well A) contains anti-Mi L chains unabsorbed; (well B) contains anti-Mi L chains absorbed with NHS and pooled lambda Bence Jones proteins; (well 1) light chains from Mi γG; (well 2) light chains from Mi γM, (wells 3 and 6) heterologous lambda light chains; and (wells 4 and 5) kappa light chains.

The light chains of the γM and γG were also compared by polyacrylamide acid gels and peptide maps. Slight mobility differences were noted by polyacrylamide gels and the peptide maps showed marked similarity with one major peptide spot difference. Both light chains contained arginine at position 190 and therefore fall into the Oz(—) group (11).

Heavy chains. The sharing of individual determinants between the whole γM and γG3 proteins in serum Mi was demonstrated initially by utilizing several antisera (type II) produced in rabbits against the electrophoretically isolated protein band containing a mixture of γM and γG. These crude antisera were rendered individually specific by absorption with pooled human serum and several γM lambda and γG3 lambda proteins. These absorbed antisera were used with highly purified preparations of the γM and γG3 proteins from serum Mi. Preliminary Ouchterlony plate analysis showed that the unre-

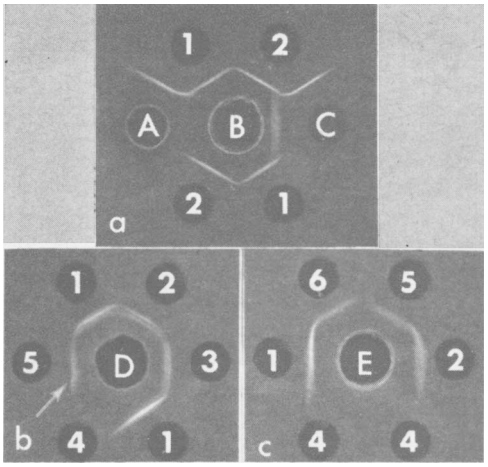

FIG. 2. Immunodiffusion analyses illustrating the shared individual specific antigens for the γG3 and γM proteins with various antisera: (a) shows the reaction of identity between the whole proteins with an antiserum made against whole isolated proteins (well B) and absorbed to give only individual specificity. Control reactions are shown with ordinary antisera to γG (well A) and γM (well C). (b) localizes the individual specificity to the heavy chains and combinational structure of heavy plus light chains utilizing an individually specific antiserum prepared against the pepsin digested γG3 protein (well D). (c) illustrates the reaction of identity between the isolated heavy chains and the intact proteins from which they were derived. The antiserum (well E) is the same as in well B with the added absorption of homologous light chains; (well 1) γG; (well 2) γM_s; (well 3) μ plus L chains; (well 4) L chains; (well 5) μ chains and (well 6) γ chains (all from Mi proteins). Note: in (b), arrow indicates precipitin dot formed by recombination of μ chains and L chains in agarose.

duced γM spurred over both the γG and reduced γM (γM_s); therefore, in order to avoid individual antigenic determinants dependent on the γM polymer, γM_s was used throughout the course of the investigation. An experiment comparing the γM_s and γG protein is shown in Fig. 2a. General antisera were utilized along with the individually specific antiserum type II. The latter antiserum was absorbed near equivalence to prevent a reaction of the excess absorbant with class specific antisera. The upper precipitin line shows identity between the class specific and individually specific precipitin reactions of the γG and γM_s proteins. The absence of precipitin lines with the class specific antisera when the contents of the wells are reversed (lower line) indicates that the observed sharing of individual determinants is not due to contamination.

The localization of these shared individual determinants was evaluated by comparing the intact γM_s and γG with the isolated heavy and light chains. It was possible to show in these experiments that some of the individual determinants were localized to the heavy chains of both the γM and γG3 protein; other shared determinants required the combination of light and heavy chains. Similar results were found with the antisera (type III) prepared against the γG3 $F(ab')_2$ fragment obtained by pepsin digestion of the γG3 protein. One experiment with a type III antiserum after absorption is shown in Fig. 2b. The isolated heavy chains from the γM protein clearly react. The light chains do not give a precipitin reaction but they combine in the agarose with the neighboring heavy chains to generate a peculiar determinant (precipitin dot at tip of arrow). This determinant is also observed as one of the double lines with the intact γM_s, γG, and recombined heavy plus light chains. This precipitin determinant is dependent on the presence of both the homologous heavy and light chains. Numerous attempts to generate this determinant by substitution of one of the chains with deterologous heavy or light chains were unsuccessful. Figure 2c shows an additional experiment with a type II antiserum illustrating the heavy chain reaction. Here, after the added absorption with homologous light chains the heavy chains give a reaction of identity with the whole γG3 and the γM_s. The specificities of the type II or type III individually specific antisera are localized primarily to the heavy chains and a unique combinational determinant of the heavy plus light chains to varying degrees with the different antisera.

Similar results were obtained with these antisera by hemagglutination techniques. The results of a typical experiment is seen in Table I. In this example the γM_s is coated to O Rh positive red cells by the bis-diazobenzidine technique. The agglutinator in

TABLE I. Hemagglutination Inhibition Experiments Showing the Cross Reaction Between the γM and γG Components of Serum Mi with an Absorbed Individually Specific Antiserum.[a]

Inhibitor	Coat: Mi γM_s; inhibitor concentration (mg/ml)							
	10.0	1.0	0.25	0.06	0.015	0.004	0.001	0.00025
1. Mi γG		0	0	0	0	0	tr	2
2. " γM_s		0	0	0	0	0	tr	2
3. " γM		0	0	0	0	0	tr	2
4. " H chains (γM)		0	0	0	0	0	1	2
5. " L chains (γG)		2	2	2	2	2	2	2
6. " L chains (γM)		2	2	2	2	2	2	2
7. Fr II + γM_s (pool)	2	2	2	2	2	2	2	2

[a] Agglutinator: Anti-Mi γM/γG mixture absorbed with NHS, heterologous γM lambda and γG3 lambda and L chains (Mi γM). Diluted 1/512 pos. = 2.

this case is a type II antiserum absorbed to render it individually specific for Mi heavy chains. The coat protein γM_s inhibited agglutination to a concentration of 0.001 mg/ml. The polymer γM and heavy chains derived from the γM inhibit to the same concentration. The homologous γG3 protein inhibits the specific hemagglutination at concentrations similar to the γM_s. In contrast, the light chains of the Mi proteins, Fr II and pooled heterologous γM_s proteins fail to inhibit the hemagglutination of the γM_s coated red cells. Similar results were obtained utilizing the γG3 as the coat protein and utilizing the heavy chain specific type III antiserum. In these experiments no individual antigenic differences either precipitating or non-precipitating could be detected between the heavy chains of the γM and γG3 proteins from patient Mi. However, in some experiments some differences were encountered for the antigens dependent primarily on H–L chain interaction.

Discussion. The serum of patient Mi contained two homogeneous immunoglobulins, one a γM lambda protein and the other a γG3 lambda protein. These proteins had the individual antigenic determinants of the light chains and heavy chains in common. Previously it had been shown by acid-urea starch gels that certain patients produce two myeloma proteins with identical light chains (12). Similar light chain identity between two homogeneous proteins in the same serum was subsequently verified by peptide mapping (13). The Mi light chains, although identical antigenically, showed slight mobility differences by polyacrylamide gel electrophoresis and sequence differences by peptide mapping. These differences which appeared to be confined to one or two amino acids of the variable region are currently under investigation.

The heavy chains showed individual antigenic determinant similarity by both hemagglutination inhibition and Ouchterlony plate analysis. Recent studies in the rabbit have shown some shared idiotypic determinants between γM and γG antibodies; these were not localized to the heavy chain, light chain, or conformational determinants (14). Another peculiar sharing of certain specific determinants has been observed with antisera against γG myeloma proteins which react with certain γM proteins in other sera (15). The explanation for this finding has not been clear. In the present report some of the individual antigenic determinants which were shared between the γM and the γG have been localized to the heavy chains. This localization was apparent since the antisera prepared against the Mi proteins gave a precipitin reaction with the heavy chains and in addition, a second precipitin reaction was generated when the light chains were recombined with the homologous heavy chains. These separate reactions proved useful in avoiding the problem of light chain contamination of some heavy chain preparations. The sharing of variable region determinants be-

tween the heavy chains of a γM and a γG strongly suggests that a segment or the entire variable region of both the μ chain and γ chain are markedly similar or identical in amino acid sequence.

The heavy chains of human immunoglobulins have been classified into four different variable region subgroups according to amino acid sequence homologies of the N-terminal segment (16). These amino acid homologies of the heavy chain have accounted for as high as 70% of all the amino acids of the variable region. The remainder of the amino acids (30%) have been attributed to antigen binding and/or individual specificity and they are located predominantly near the C-terminal end of the variable region. Therefore, because of the uniqueness of individual antigenic determinants it may be postulated that Mi μ and Mi γ chains probably have similar amino acid sequences in the area of maximum amino acid sequence randomness. This postulate is reasonable since it has recently been demonstrated that antibodies in an individual rabbit (both γM and γG) prepared against a hapten have similar amino acid composition of the C-terminal segment of the variable region (17).

The sharing of individual antigenic determinants between the μ and γ chains, however, may also indicate that the entire variable regions of both types of heavy chains are identical. Studies on the amino acid sequence homologies which are currently in progress will be required to answer this question conclusively. If this is the case, it would indicate that the same gene can code for the variable area of different heavy chains.

It is known that the constant areas of these heavy chains are under independent genetic control (18). These interpretations extend to those made previously on the two-gene control of heavy chains on the basis of the finding of similar variable region subclasses on γG and γM proteins (5). The mechanism and level at which linkage occurs for the formation of one heavy chain remains obscure.

Additional examples of the situation found for serum Mi certainly exist. Preliminary studies have been made on two other sera and the same principles appear operative. One of these is an example of a γG–γA double band. A preliminary report on the sharing of individual specificity in the case of another γG–γM combination by Hopper *et al.* (19) has appeared along with an earlier report of the present study (20).

Summary. Two lambda proteins, one a γM and the other a γG3, were isolated from the same human myeloma serum and were shown to share individual antigenic (idiotypic) specificity when tested with several antisera prepared against the intact proteins and pepsin digested fragments. These shared determinants were localized to the heavy chains and the combinational structure of the heavy plus light chains. Shared individual determinants of the light chains were also demonstrated utilizing antisera prepared against the isolated light chains. These results indicate that the light chains and the variable regions of the heavy chains of the two proteins are extremely similar. The peptide map and mobility differences in the light chains indicate that they are not identical.

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