COMMON INDIVIDUAL ANTIGENIC DETERMINANTS IN FIVE OF EIGHT BALB/c IgA MYELOMA PROTEINS THAT BIND PHOSPHORYL CHOLINE

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Recently a large number of myeloma proteins derived from plasma cell tumors induced in the inbred BALB/c strain of mice have been tested for their ability to bind antigens. The first active protein found was a serum IgA myeloma protein that precipitated with the Pneumococcus C polysaccharide (1). Since this original report, seven more IgA proteins, each derived from an independently induced tumor, have been found which precipitate with the Pneumococcus C polysaccharide (2, 3, and this paper). Of the eight tumors, two originated in the laboratory of Dr. Melvin Cohn in La Jolla and six in our laboratory in Bethesda.

The Pneumococcus C polysaccharide is a complex antigen whose repeating unit contains galactose, N-acetyl galactosamine, glucose, 2-acetamido-4-amino-2,4,6-trideoxyhexose, ribitol, phosphate (4, 5) and choline (6). Recently Leon and Young have shown that the IgA myeloma proteins that bind the Pneumococcus C polysaccharide apparently all react with the same haptenic group on this antigen, as they have been able to inhibit precipitation of the Pneumococcus C polysaccharide by the IgA myeloma proteins with phosphoryl choline (7).

We have recently found another antigen derived from a species of *Lactobacillus acidophilus* that is also precipitated by these proteins. This *Lactobacillus acidophilus* species is a common normal inhabitant of the BALB/c mouse's gastrointestinal tract.

Interesting questions involving a functionally related group of homogeneous immunoglobulins derived from genetically similar hosts (inbred BALB/c strain mice) concern similarities and differences in structure among the individual proteins. In a previous report, differences in two proteins in this group were reported (2). The question we wish to consider in this paper is whether similarities or even identities exist among any of the eight proteins in this group.

Similarities must ultimately be established by comparison of the primary structures of the light and heavy polypeptide chains. However, another sensitive method for determining similarities among immunoglobulins of monoclonal origin (M-proteins) is by demonstrating common individual antigenic specificities. Many M-proteins have been shown to possess individual or M-specific determinants (MSD)[1] that are unique

[1] *Abbreviations used in this paper:* MSD, M-specific determinants; PBS, phosphate-buffered saline, pH 7.4.

to the individual protein used for immunization. Antisera specific for individual homogeneous immunoglobulins (M-proteins) have been prepared for both human (8) and mouse (9, 10) M-proteins.

Several series of functionally related M-proteins, the IgM cold hemagglutinins (11, 12), the IgG antistreptolysins (13) in man, and the IgA antilipopolysaccharide precipitins in the mouse (14) have already been studied using myeloma or M-specific antisera. Only with the cold hemagglutinins have common (cross-specific) determinants been found on immunoglobulins of different cellular origin, and these occurred only on IgM proteins with cold hemagglutinating ability and not on IgM immunoglobulins (Waldenström macroglobulins) lacking this property. This result suggests that some cold agglutinins possess common structures. The serologic findings, however, indicated that while individual specificities were shared by different IgM cold agglutinins, these proteins often differed by other criteria, e.g. In antigen-binding specificity or in their light chain subunits. Allotypic differences may further mask important individual antigenic specificities. Whether two human IgM-cold agglutinins are identical has neither yet been proven nor ruled out for cold hemagglutinins of independent cellular origin.

In this study we have prepared homologous antisera to each of the eight IgA myeloma proteins that bind phosphoryl choline. We have found that three of the proteins have their own unique MSD and that five have a common MSD and thus far lack further individual characteristics. These findings have suggested the possibility that the five IgA proteins (H8, T15, S63, S107, and M299) are extremely similar. Previously Cohn et al. have reported a common MSD on the two IgA proteins S63 and S107 which originated at the Salk Institute (3).

Materials and Methods

Source of Plasma Cell Tumors.—The plasma cell tumors that produce IgA-class myeloma proteins with anti-phosphoryl choline-binding activity were induced in BALB/c mice by the intraperitoneal injection of mineral oil, 2,6,10,14-tetramethylpentadecane (Pristane), or 7-N hexyloctadecane (Table I) (15, 16). Each of these saturated hydrocarbons was injected in 0.5 ml amounts intraperitoneally three times when the mice were 2, 4, and 6 months of age. Two of the tumors, S63 and S107, were induced by Dr. Melvin Cohn of the Salk Institute (1, 3) and sera from these mice were kindly provided by him for this study.

Myeloma Protein Preparation.—The plasma cell tumors were converted to the ascites form by intraperitoneal passage of the tumor cells. Usually after one to two transfer generations the tumors produced the ascites in the new hosts. Groups of 20–30 mice were inoculated with ascites tumor cells and when the ascites formed, the fluid was harvested by paracentesis with a sterile 19 gauge needle. Usually two to three collections per mouse were possible. The ascites was diluted 1:2 with 0.005 M sodium phosphate buffer containing 0.15 M NaCl (PBS [phosphate-buffered saline]). The myeloma proteins were then precipitated from diluted serum or ascites at 4°C by adding saturated ammonium sulfate. Some IgA myeloma proteins are readily precipitated at 33–37% saturation, others require 50% saturation with ammonium sulfate. The proteins as a rule were solubilized in PBS and reprecipitated a second time at 37–40% saturation in ammonium sulfate. The resolubilized precipitate was then dialyzed against water

and the euglobulin fractions separated from the supernate. As a rule the euglobulin fractions contained aggregated polymers which usually can be resolubilized in 0.2–0.4 M NH_4HCO_3. The euglobulin or water-soluble fractions were used as antigens in most of the studies. These fractions are identified as polymer fractions.

Preparation of Fab.—Polymer fractions were reduced in 0.03 M dithiothreitol (DTT) for 1 hr at room temperature and S-carboxy-methylated with 0.066 M iodoacetamide in 0.5 M tris(hydroxymethyl)aminomethane (Tris) chloride at pH 8.6 for 15 min. This converts the polymer to a monomeric 5.9 S form. Monomeric IgA was digested with mercuripapain (Worthington Biochemical Corp., Freehold, N. J.) in PBS with a protein to enzyme ratio 16:1 for 1 hr at 37°C. DTT and EDTA were used to activate the papain and after digestion the mixture was treated with iodoacetamide to inactivate the essential sulfhydryl groups in the active site of papain. Agar gel electrophoresis and serologic studies revealed that Fc fragments (which carry the $A^{12, 13, 14}$ allotypic markers) were destroyed by this procedure. Some Fab fragments were further purified by ion-exchange chromatography on A-25 DEAE Sephadex (Pharmacia Fine Chemicals, Uppsala, Sweden).

Ultracentrifuge.—The M603 monomer was ultracentrifuged in the Model L analytical Spinco ultracentrifuge (Beckman Instruments, Fullerton, Calif.) at a concentration of approximately 10 mg/ml. Similarly, the Fab fragments of M603 and T15 were also run in the ultracentrifuge. The sedimentation coefficients of the monomeric IgA were found to be 5.9 $S_{20}W$. Sedimentation coefficients were determined on both chromatographically purified Fab and dialyzed papain digests of IgA monomers. Both preparations consisted of a single peak in the ultracentrifuge with a sedimentation coefficient of 3.54 S. Following papain digestion some protein is converted to a relatively insoluble or aggregated form. A small 11 S peak was noted in the monomer preparations.

Lactobacillus Antigens.—A species of *Lactobacillus acidophilus* isolated from the normal intestinal flora of BALB/c mice in our colony contained an antigen that is also precipitated by the eight myeloma proteins used in this study. The antigen was originally isolated by trichloroacetic acid extraction of pressure-disrupted *Lactobacillus acidophilus* strain 4 by Mr. Charles K. Mills of the American Type Culture Collection. In the present experiments, this antigen was isolated from the species of *Lactobacillus acidophilus* by the method of Gotschlich and Liu (4). Essentially Lactobacilli were lysed in deoxycholate and then the treated organisms were heated in a boiling water bath for 30 min. The antigen was extracted by precipitation with 95% ethyl alcohol.

Inhibition of Precipitation by Phosphoryl Choline and Choline.—Antigenic determinants identified in the *Lactobacillus acidophilus* antigen and Pneumococcus C polysaccharide are probably similar since the precipitation of both antigens by the eight IgA myeloma proteins in this study is inhibited by phosphoryl choline. Quantitative inhibition of precipitation of Pneumococcus C polysaccharide with the IgA myeloma proteins by phosphoryl choline, choline, and other related compounds had been described originally by Leon and Young (7). In the present study we have employed a different method of inhibition of precipitation using agar gel methods and the Lactobacillus antigen instead of Pneumococcus C polysaccharide. The IgA myeloma proteins were reacted with the Lactobacillus antigen in double diffusion agar gels. After the precipitin lines had formed the gels were then immersed in solutions containing the inhibitors. Inhibition of precipitation was indicated by the disappearance of the precipitin line.

Immunization of Inbred Mice with IgA Myeloma Proteins.—Each mouse was injected intraperitoneally and subcutaneously in five areas including the two footpads, two axillary regions, and in one trunk area region with 75 μg of the myeloma protein in 0.1 ml of equal parts of physiologic saline and complete Freund's adjuvant. The same injection in the identical areas was repeated 3–4 days later except that incomplete Freund's adjuvant was used. The pro-

cedure was then repeated twice at 3–4 day intervals in the same areas with the myeloma protein alone. The mouse was first bled 4 wk after the first injection from the infraorbital sinus, and the serum was tested for precipitating antibody in double diffusion agar plates. If no precipitins were found, similar injections were given with the myeloma protein alone at weekly intervals. Most precipitins were usually detected in 4 wk.

RESULTS

Source and General Characteristics of the Plasma Cell Tumors.—The source of the eight plasma cell tumors is given in Table 1. The two tumors, S63 and S107,

TABLE I

Source of the Anti-Phosphoryl Choline–IgA-Producing Plasma cell Tumors; Light Chain Classes

Tumor source of myeloma protein*	Material used to induce tumor	Source, reference	Location	Year	Light chain	
					Class	Subclass
MOPC 167	Bayol F	Potter (2)	Bethesda	1963	κ	IX
MOPC 299	Bayol F	Potter (2)	"	1966	κ	—
McPC 603	Bayol F	McIntire (2)	"	1965	κ	III
TEPC 15	Pristane	Anderson (16)	"	1969	κ	VI
HOPC 8	7 N-hexyloctadecane	Anderson (16)	"	1969	κ	VI
MOPC 511	Bayol F	Potter‡	"	1969	λ	—
S 63	Mineral oil	Cohn (3)	La Jolla	1967	κ	—
S 107	Mineral oil	Cohn (3)	"	1967	κ	—

* The myeloma protein will be designated by the first letter and number corresponding to the tumor source. The myeloma protein derived from MOPC 167 = M167. Abbreviation of tumor source is based on material used to induce tumors (MOPC = mineral oil plasma cell) or name of investigator that induced tumor (McPC = McIntire plasma cell) or name of Institute where tumor was induced (S = Salk) TEPC is an abbreviation for tetramethylpentadecane plasma cell tumor. HOPC is an abbreviation for 7-N-hexyloctadecane.

‡ This paper.

have previously been described by Dr. Melvin Cohn and associates (1, 3) and we have used them only in serologic studies, i.e., the tumors have not been maintained at any time in this laboratory. Three of the tumors in our collection, M167, M299, and M603 have been previously described (2). The MOPC 299 tumor lost its protein-producing ability in 1967 and all of the studies have been made with a few available serum samples taken during its early transplant history. Three other tumors, TEPC 15, HOPC 8, and MOPC 511, are described here for the first time. The TEPC 15 and HOPC 8 plasma cell tumors were induced in separate experiments by Dr. Paul Anderson at the National Cancer Institute. Precipitation with the Pneumococcus C polysaccharide by these myeloma proteins was discovered during a routine screening

procedure against a series of antigens. The H8 and T15 are kappa type IgA-producing tumors not associated with urinary protein production. The M511 tumor, by contrast, has a rare lambda type light chain subunit and is associated with Bence Jones proteinuria.

Inhibitions of Precipitation by Phosphoryl Choline and Choline.—All eight myeloma proteins in this study precipitated with the Lactobacillus antigen. Precipitation of six of these proteins (those originating in our laboratory) with Lactobacillus antigen is shown (Fig. 1, left). Lines of identity are formed with five of the proteins that have the kappa type light chains and a line of partial identity is formed with the 511 (lambda chain-containing protein) with adjacent kappa type IgA myeloma proteins. In the inhibition of precipitation

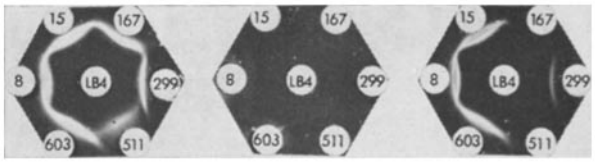

FIG. 1. Ouchterlony reactions showing inhibition of precipitation of Lactobacillus antigen LB4, with phosphoryl choline and choline. Precipitin bands were allowed to develop overnight and then the gels were immersed in the inhibitor or solution. The control reaction before inhibition is Fig. 1, left; Fig. 1, center is the same gel after its being immersed in 0.001 M phosphoryl choline for about 60 min. On the right is a third plate similar to the control Fig. 1, left, which was immersed in 0.01 M choline chloride. The IgA myeloma proteins were polymer fractions for all of the proteins except M299, for which whole serum was used; the concentration of myeloma protein was 8 mg/ml for H8, T14, M603, and M511. Concentration for M167 was 24 mg per ml. The Lactobacillus antigen was a 1 mg/ml solution of an alcohol-precipitated extract from deoxycholate-lysed cells. The phosphoryl choline removes all the precipitins while the choline chloride removes only those to M167 and M511.

studies, the precipitin bands produced with the IgA myeloma proteins and the Lactobacillus antigen were removed by 0.001 M phosphoryl choline (Fig. 1, center). With the 0.01 M choline chloride, however, the precipitin bands of only the M167 and M511 with Lactobacillus antigen were removed (Fig. 1, right). These results agree with the observations of Leon and Young (7) who demonstrated that the M167 was quantitatively inhibited by free choline chloride in contrast to M299, M603, and T15, which were not inhibited at these and 100-fold higher concentrations of choline chloride. It was concluded by Leon and Young that the M167 combining site did not require the presence of the phosphoryl group if the hapten was to be bound (7).

Specificity of Antisera Prepared to IgA Myeloma Proteins that Bind Phosphoryl Choline.—Homologous antisera were prepared to all eight myeloma proteins (Table II). Inbred strain NH, CE, A/He, and AL mice were used as recipients for immunizations. M-specific antisera were successfully prepared in

different strains by immunization with the same myeloma protein. Some of these same antisera also identified allotypic specificities, but these could be made specific for MSD by absorption with the proper IgA Fc determinants. In this study only antisera specific for MSD were employed.

The antisera prepared to M167, M511, and M603 were specific for the respective immunizing antigen (Fig. 2, left). Each of these proteins contained an individual determinant or MSD that was not found on any of the other BALB/c IgA anti-phosphoryl choline proteins nor on over 120 other myeloma

TABLE II

Myeloma-Specific Antisera

Immunizing antigen	Recipient strain	Number of mice		
		Immunized	Producing antisera to	
			MSD	Allotypic + MSD
M511	NH	3	1	0
	CE	1	1	0
	AL	6	3	0
M167	AL	4	3	1
	A/He	4	3	1
M603	A/He	8	8	0
	CE	6	4	2
M299	A/He	4	4	0
	AL	3	3	0
T15	A/He	4	4	0
	AL	4	4	0
H8	A/He	8	8	0
S63	A/He	4	4	0
	NH	6	1	0
S107	A/He	6	1	0
	NH	6	1	0

proteins from the BALB/c mouse including 80 IgA proteins which were tested (Table III).

The antisera prepared to H8, T15, S63, S107, and M299 behaved differently for each one of these antisera precipitated not only with the immunizing antigen but with all of the five myeloma proteins mentioned (Fig. 2, right). This was a particularly surprising result since myeloma proteins developed in two different laboratories (e.g. S63 and S107 from Dr. Cohn's laboratory and H8, T15, and M299 from our laboratory) shared a common MSD. This shared MSD was specific for only this group of proteins and was not found on a large series of other myeloma proteins tested (Table III).

Localization of Myeloma-Specific Determinants.—The polymeric IgA mye-

loma proteins were reduced in 0.03 M DTT at pH 8.9 and S-carboxy-methylated with iodoacetamide. This procedure converts the polymers to a monomeric 5.9 S form. Five of the myeloma proteins (H8, T15, M167, M511, and M603) were converted to 5.9 S and tested with the appropriate MSD anti-

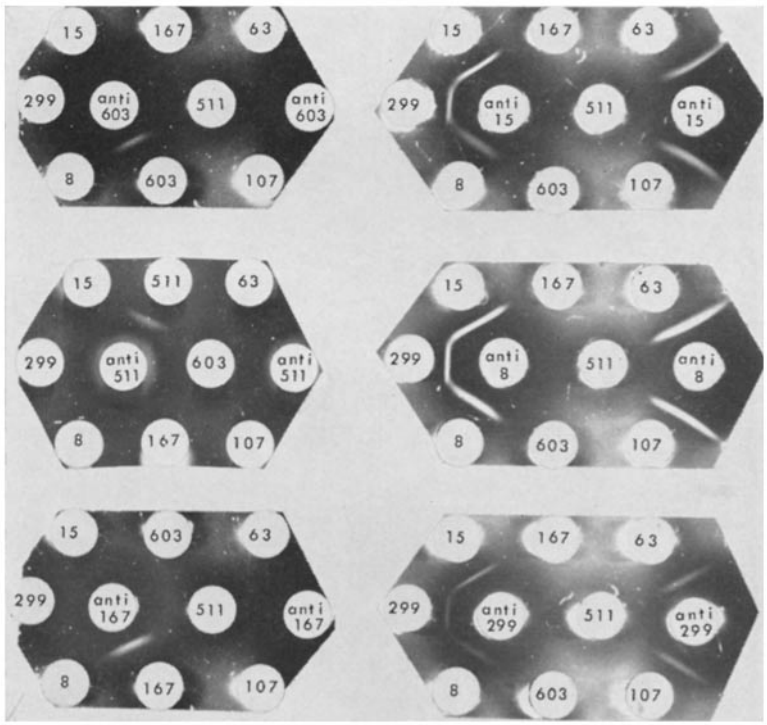

FIG. 2. Ouchterlony reactions with myeloma-specific antisera. Antisera to myeloma proteins were prepared in A/He, except M511 which was in AL. Each antiserum was reacted with all eight of the IgA myeloma proteins that bind phosphoryl choline-containing antigens. The anti-M603, anti-M511, and anti-M167 antisera react only with the respective immunizing antigen. The anti-T15, anti-H8, and anti-M299 antisera all react with five proteins: H8, M299, T15, S63, and S107.

serum (Table IV). Each of the antisera precipitated with 5.9 S monomer (see example in Fig. 6).

IgA myeloma proteins in the mouse are readily cleaved by papain into Fab and Fc fragments. When polymeric IgA molecules are used Fc fragments are obtained but when the monomeric (5.9 S) form is digested, the Fc fragments are usually destroyed. In the present experiments, papain Fab fragments of H8, T15, M167, M511, and M603 monomers were prepared. These Fab frag-

ments were tested with an anti-allotypic IgA antiserum which identifies the A^{12} determinant located on the Fc part of the molecule. This antiserum failed to precipitate with the Fab fractions indicating, destruction of the Fc fragment (Fig. 3).

TABLE III

Summary of Agar Gel Precipitin Reactions with Myeloma-Specific Antisera

Antisera		Anti-phosphoryl choline myeloma proteins								Number of negative myeloma proteins tested			
Immunizing antigen	Recipient strains	H8	T15	S63	S107	M167	M299	M511	M603	IgA	IgF	IgG	IgH
M167	AL, A/He	−	−	−	−	+	−	−	−	84	19	11	15
M511	AL, CE	−	−	−	−	−	−	+	−	83	16	9	10
M603	A/He, CE	−	−	−	−	−	−	−	+	84	19	11	15
H8	A/He	+	+	+	+	−	+	−	−	77	16	9	10
T15	A/He, AL	+	+	+	+	−	+	−	−	64	16	9	9
M299	A/He, AL	+	+	+	+	−	+	−	−	77	15	7	9
S63	A/He	+	+	+	+	−	+	−	−	60	15	8	9
S107	A/He	+	+	+	+	−	+	−	−	77	15	7	9

IgF = γ1; IgG = γ2a; IgH = γ2b.

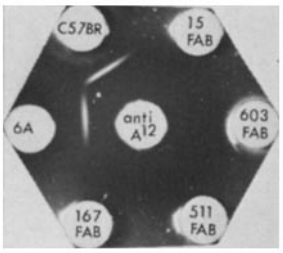

FIG. 3. Ouchterlony reactions in which an IgA myeloma allotypic antisera (anti-A^{12}) was reacted with the IgA myeloma protein (Adj. PC-6A), normal serum from strain C57BR, and four different IgA myeloma Fab fragments. Strong precipitin lines were obtained with the purified IgA myeloma protein 6A and the normal serum from C57BR, but not with the four Fab fragments. The allotypic antiserum was prepared in strain DE by immunization with MOPC 209 IgA myeloma protein.

The T15 and M603 Fab fragments were ultracentrifuged to determine homogeneity (Fig. 4) and the ultracentrifuge runs revealed only a single component with an $S_{20}W$ coefficient of 3.54 S (Fig. 4). The antisera prepared to M167, M511, M603, and H8 did not precipitate with their Fab fragments (Table IV). It has been noted previously, however, that Fab fragments inhibit precipitation of a myeloma protein with its M-specific antiserum (9). This inhibitory effect is demonstrated in agar gels by the failure of precipitin

lines to extend into regions where Fab is in high concentration (Fig. 5). The explanation for this phenomenon is that the Fab fragments are antigenically univalent and form soluble complexes with the MSD antisera, and these soluble complexes block the extension of precipitin bands. Fab fragments,

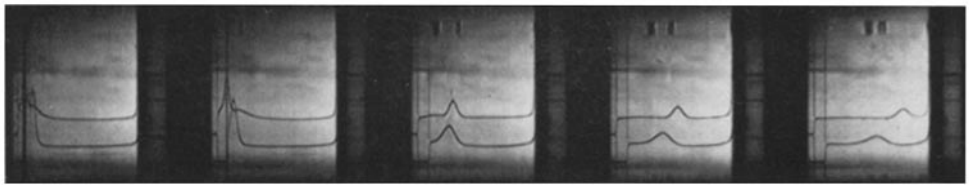

FIG. 4. Ultracentrifuge studies of T15 monomer (upper) and M603 Fab (lower). The proteins were centrifuged at 59,780 rpm; the photographs were taken at 15, 31, 47, 63, and 79 min. A small shoulder in advance of the major peak is seen for the monomer in the 15- and 31-min photograph. The S_{20} coefficient for the monomer was calculated to be 5.92 S while the sedimentation coefficient for the Fab was 3.54 S.

TABLE IV

Absorption of Myeloma-Specific Antisera with Fab Fragments

Antiserum to	Absorbant	IgA Polymer								Fab					5.9 S Monomer				
		8	15	63	107	167	299	511	603	8	15	167	511	603	8	15	167	511	603
M167	0	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−
	M167 Fab	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	M603 Fab	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−
M603	0	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
	M167 Fab	−	−	−	−	−	−	−	+	−	−	−	−	−					
	M603 Fab	−	−	−	−	−	−	−	−	−	−	−	−	−					
M511	0	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−
	M511 Fab	−	−	−	−	−	−	−	−	−	−	−	−	−					
H8	0	+	+	+	+	−	+	−	−	−	−	−	−	−	+	+	−	−	−
	H8 Fab	−	−	−	−	−	−	−	−	−	−	−	−	−	−				
	T15 Fab	−	−	−	−	−	−	−	−	−	−	−	−	−	−				
T15	0	+	+	+	+	−	+	−	−	+	+	−	−	−	+	+	−	−	−
	H8 Fab	−	−	−	−	−	−	−	−	−	−	−	−	−	−				
	T15 Fab	−	−	−	−	−	−	−	−	−	−	−	−	−	−				
BALB/c IgA allotypes		+	+	+	+	+	+	+	+	−	−	−	−	−					

however, carry the M-specific determinants; absorption of the anti-H8 and anti-T15 M-specific antisera with either H8 or T15 Fab fragments (Fig. 6) and also the other M-specific antisera with their respective Fab fragments completely removes all the precipitin reactions (Table IV).

The antiserum to T15 was exceptional in that it precipitated with its Fab fragment as well as with the H8 Fab fragments (Table IV, Fig. 7, left). The precipitin lines formed with the Fab fragments showed only partial identity with

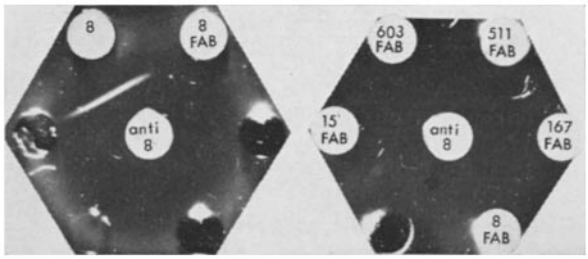

FIG. 5. Ouchterlony reactions. On the left the anti-H8 antiserum precipitates with the H8 polymer, but not with the H8 Fab fragment. Note that the precipitin lines with the polymer extends into the well on the left but that the precipitin line does not extend an equal distance towards the source of the H8 Fab fragment, indicating inhibition of growth of the precipitin lines. The reaction on the right shows the failure of anti-H8 to precipitate with five different Fab fragments.

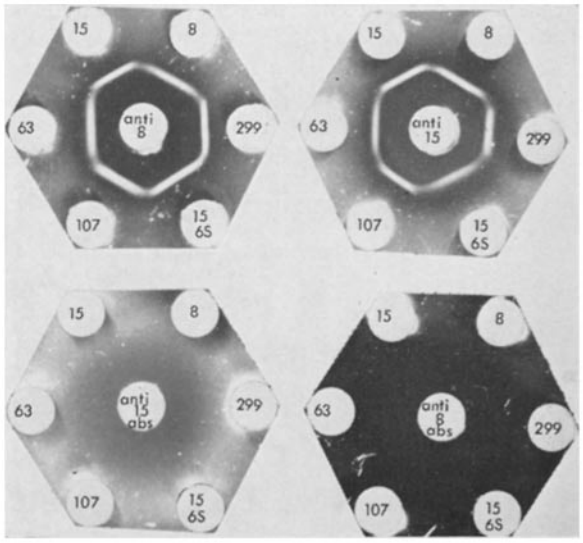

FIG. 6. Effect of absorption of anti-H8 and anti-T15 antisera with T15 and H8 Fab fragments respectively. Upper left and upper right show precipitation of five different polymer IgA myeloma proteins, H8, T15, S63, S107, and M299, with the anti-H8 and anti-T15 antisera. Also included is the ability of each of these antisera to precipitate with the T15 monomer (labeled T15 6 S). Following absorption all precipitin lines were removed.

those produced to the respective IgA polymer or monomer (Fig. 7, right). Furthermore, even though a precipitin band formed with anti-T15 and T15 Fab, the Fab inhibition effect was still observed by shortening of the neighboring precipitin bands (Fig. 7, center). These results indicate that the anti-T15

antiserum recognizes two topographically different determinants, both of which are present on the H8 and T15 Fab fragments. These two sites are far enough apart to permit simultaneous binding of the Fab fragments by more than one antibody molecule to form a precipitate. This finding is of considerable importance for it provides additional evidence of the similarity of the H8 and T15 proteins.

Experiments on the localization of the M-specific determinants to specific chains or to conformations controlled by chain interactions are in progress.

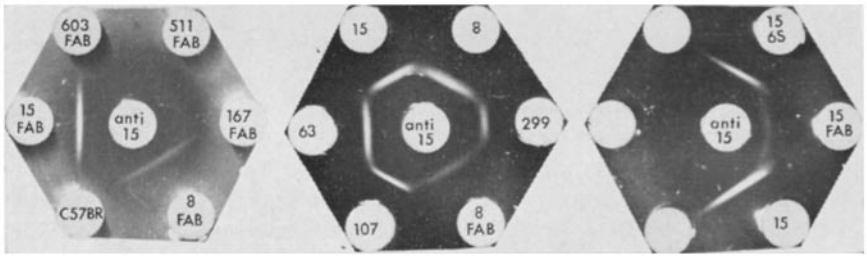

FIG. 7. Ouchterlony reactions demonstrating characteristics of the anti-T15 myeloma-specific antiserum. On the left the anti-T15 antiserum precipitates with the H8 Fab fragment and the T15 Fab fragment but not with three other Fab fragments nor with C57BR normal serum. In the center the anti-T15 MSD antiserum precipitates with five different IgA myeloma proteins, H8, T15, S63, S107, and M299, and also the H8 Fab fragment. Note the inhibition of precipitation of the S107 and M299 lines in the regions where H8 Fab fragments are in high concentration. This produces a distorted hexagon. Reaction on the right shows the precipitation of anti-T15 with the T15 Fab, with T15 polymer, and with T15 5.9 S (6S). Note again the striking inhibition of precipitation in the regions where T15 Fab is most highly concentrated. Note also the line of partial identity of the T15 Fab with the monomer and polymer precipitin lines.

DISCUSSION

The extraordinary specificity of the monoclonal-specific antisera for the immunizing M-protein antigen suggests that the anti-M167, anti-M511, and anti-M603 antisera can precipitate with only one species of immunoglobulin molecule. The same degree of specificity has been noted for anti-idiotypic antisera (antisera specific for conventionally raised antibodies) (17–19). One explanation for why anti-idiotypic antisera can be produced is that the antibody used in immunization in fact contains a predominating homogeneous component in sufficient concentration to induce M-specific antibodies (19). It seems, therefore, safe to conclude that one characteristic of a homogeneous or monoclonal immunoglobulin is its unique antigenicity. The specificity of M-specific antisera produced in mice (homologous immunization) appears to

be even greater than that from heterologous immunization since species differences are not involved in the immunization which could obscure M-specific determinants.

The antisera that precipitate with the group of anti-phosphoryl choline IgA M-proteins (H8, T15, S63, S107, and M299) are also highly specific for the group. These antisera also failed to precipitate with many other BALB/c IgA, IgF, IgG, and IgH M-proteins (Table III). This common or shared specificity resembles the M-specificities assigned to single proteins. Two possible explanations for the common M-specific determinants are (*a*) the H8, T15, S63, S107, and M299 proteins are structurally identical or (*b*) the H8, T15, S63, S107, and M299 share a common cross-specific antigenic determinant but nonetheless possess structural differences which are not apparent. We have been able to produce an M-specific antiserum to every IgA myeloma protein tested thus far, and for this reason the first explanation appears more plausible to us at this time.

Structural studies currently support this view. In experiments conducted in collaboration with Dr. Leroy Hood, National Cancer Institute, (to be reported separately) on the amino-terminal sequences of the H8 and T15 light chains, as well as on tryptic and thermolysin peptide maps, indicate the identity of H8 and T15 kappa light chains. Amino-terminal sequences and tryptic peptide maps of the M167 and M603 kappa chains reveal multiple differences from one another as well as from H8 and T15 (2, 20). Thus IgA M-proteins that bind phosphoryl choline antigens may be structurally very different from each other. The structural and antigenic similarities of H8 and T15 thus far demonstrated appear to predict that as the analysis progresses identity may indeed be established. Furthermore, the structural identities of H8 and T15 lead one to predict that the S63 and S107 proteins will also be identical to H8 and T15.

Five identical myeloma proteins of independent cellular origin suggest that the same genes controlling light and heavy chains have been differentiated in the different cells, i.e., genetically controlled identical antibodies have developed independently in five different mice.

Four types among the eight IgA myeloma proteins that bind phosphoryl choline are clearly different from one another. Each of these proteins appears to resemble a single molecular species of immunoglobulin and hence might be analogous to homogeneous antibody. It is generally thought that the basis of the immune mechanism depends upon selection of predetermined or predifferentiated immunocytes by antigens which stimulate them to undergo further development and transformation to plasma cells. Finding four different molecular species that bind the same hapten in different mice suggests that antigens containing phosphoryl choline have exerted a specific selective influence on the total population of immunocytes in BALB/c mice during plasma cell tumor formation. A natural source of phosphoryl choline-containing antigen

in the mouse is of course the Lactobacillus antigen which is derived from the *Lactobacillus acidophilus* species that is prevalent in the gastrointestinal tract of BALB/c mice. Other sources of phosphoryl choline-containing antigens available in the mouse might be the intestinal helminths; it is known that extracts of ascaris also contain phosphoryl choline-containing antigens (15). A third potential source of phosphoryl choline-containing antigens are the lecithins and sphingomyelins which are found in membranes of animal tissues. Thus the source of antigenic stimulation to precursors of neoplastic plasma cells could then be postulated to be of autogenous origin.

Lipids usually are not immunogenic. The production of anti-lipid antibodies depends upon coupling lipids to antigenic protein carriers (21). However the bacterial antigens, such as Pneumococcus C polysaccharide and Lactobacillus antigen, may be highly immunogenic because of additional antigenic groupings, and may sensitize the host animal to the chemical group that is prevalent in the host, i.e., phosphoryl choline. Whatever the source of antigens that selects the cells that produce anti-phosphoryl choline type immunoglobulins must be generally distributed in mice to account for the frequent appearance of tumors that produce this type of specific immunoglobulin molecule.

SUMMARY

Eight IgA myeloma proteins derived from independently induced plasmacytomas in genetically similar inbred BALB/c mice are functionally related by their binding of phosphoryl choline-containing antigens (Pneumococcus C polysaccharide or Lactobacillus antigen). Each protein resembles a single species of immunoglobulin in antibody. The proteins are characterized by highly sensitive myeloma-specific antisera prepared by immunizing mice of other inbred strains with the BALB/c myeloma proteins. Individual or myeloma-specific determinants located on Fab fragments were found on three of the proteins that were unique for that protein and did not react with any other IgA protein among over 70 tested. Remarkably, five of the proteins shared two common myeloma-specific determinants which were specific for this group of five proteins. These results suggest that the five functionally and genetically related proteins sharing the same myeloma-specific determinants might also be structurally similar.

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