IMMUNOGLOBULIN ISOANTIGENS (ALLOTYPES) IN THE MOUSE

II. Allotypic Analysis of Three γG_2-Myeloma Proteins from (NZB × BALB/c)F_1 Hybrids and of Normal γG_2-Globulins*

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Genetically controlled isoantigenic differences (allotypes) of 3 of the immunoglobulin classes of mice have recently been demonstrated (1–7). These antigenic polymorphisms provide useful markers in studies of protein synthesis and some aspects of somatic cell genetics (8). Myeloma proteins produced by plasma cell tumors have been particularly useful in defining the structure, subdivisions, and genetic control of immunoglobulin molecules (9–11).

The induction of plasma cell tumors in mice has been reported by several groups (12, 13) using prolonged treatment of mice with mineral oil or other agents. These tumors have usually been produced in BALB/c mice with only the occasional tumor reported in C3H, CBA, DBA/2, or hybrids of these strains (13).

A new series of transplantable plasma cell tumors has recently been developed in hybrid mice of the cross NZB × BALB/c (14). The antigenic classification (as defined by Fahey et al., 15 and 16) of the myeloma proteins of 8 plasma cell tumors produced in these hybrid mice has previously been reported. It was shown that the 8 lines comprised 4 γA-, 1 γG_1-, 1 γG_{2b}-, and 2 γG_{2a}-myelomas.

Some human myeloma proteins carry the isoantigens (Gm and InV) (10, 17–19) of normal human immunoglobulins. These proteins carry the Gm type of only one allele even from patients heterozygous at this locus (20–22). In instances where more than one specificity of a Gm allele has been defined, myeloma proteins can carry these several specificities (22–24).

We have undertaken a detailed isoantigenic analysis of the 3 γG_2-myelomas produced in (NZB × BALB/c)F hybrid mice, in order to see whether (*a*) the

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myeloma protein carries isoantigenic specificities of one or both of the parental strains, and (*b*) it carries all of the isoantigenic specificities normal to its class of immunoglobulin.

Materials and Methods

Plasma Cell Tumors.—The origin of the transplantable plasma cell tumors GPC-5, GPC-7, and GPC-8 has been described (14). Each of these plasma cell tumors was produced and passaged in (NZB × BALB/c)^{1}F$_1$ hybrid mice. Sera were obtained from the original hosts of GPC-7 and GPC-8 and from a first passage recipient of GPC-5. The serum samples were preserved with 0.1% sodium azide and sent air mail from Melbourne to Palo Alto where they were kept at −20°C until analysis.

Myeloma proteins from previously established lines were isolated for use as standard antigens and inhibitors. Tumors RPC-5, (adj PC-5), MPC-1, MPC-11, MPC-31 were passaged in BALB/c mice; 5563 was passaged in C3H mice (15).

Nomenclature of Immunoglobulins.—The nomenclature for immunoglobulin classes proposed (25) by the World Health Organization Committee on nomenclature of human immunoglobulins has been followed. The 5 immunoglobulin classes of mice defined by Fahey et al., (15, 16) are here called γM, γA, γG_1, γG_{2a}, γG_{2b}. We have changed 7Sγ_1 to γG_1 etc., in order to more closely follow the World Health Organization Committee recommendation.

An extension of this system for the γG-globulins of mice is under consideration and will be followed when adopted. The isoantigenic(allotypic) loci for γG_{2a} and γA have previously been named Ig-1 and Ig-2. In this paper Ig-3 is the designation for the allotypes appearing on γG_{2b}-molecules. Specificity designation is made following the proposal of Snell et al. (26) for the H-2 locus; e.g., for C3H γG_{2a}-isoantigenic specificities, Ig-1. 1, 2, 6, 7, 8, 9, 10.

Isolation of Myeloma Proteins—. Zone agar gel electrophoresis of 50 μl samples of sera from GPC-5, GPC-7, and GPC-8 was performed in 1% Ionagar (Oxoid Division, Consolidated Laboratories, Chicago Heights, Illinois) in veronal buffer pH 8.2 on glass plates at 6 to 8 v/cm, 30 ma for 40 to 60 min. Serial 5 mm strips were then cut perpendicular to the direction of current flow and protein was extruded from these strips by centrifugation at 100,000 × *g* for 1 hr. Protein determinations on the extruded fractions revealed a typical myeloma spike in the slow cathodal region. Portions of the fractions containing these myeloma spikes were then iodinated or used as inhibitors. The contamination of these 3 fractions with normal gamma globulins is about 5% (as determined by inhibition assays for isoantigens not carried on the myeloma protein).

Isoantigenic Analysis.—Precipitation of iodinated antigens, inhibition of precipitation of iodinated antigens, and the production of antiallotype sera, have been previously described (6). Briefly, the slow gamma globulins from a normal or myeloma-containing serum are purified by ammonium sulphate precipitation, zone electrophoresis, and Sephadex gel filtration, and then conjugated with I^{125}- by the chloramine-T method. Fifty μl portions of appropriate dilutions of a labeled antigen in 3% BSA in 0.05 M tris buffer, pH 7.6 are pipetted into 6 × 50 mm tubes. To each of these is added 5 μl of a dilution of either a purified protein antigen (the standard of known protein concentration) or a dilution of serum from normal or the plasma cell tumor-bearing mice. Each tube then receives 50 μl of S-dil (3% BSA and 10% normal rabbit serum in 0.05 M Tris pH 7.6) containing a constant amount of antiallotype serum (usually an amount previously shown to precipitate about 50% of the labeled antigen in the absence of inhibitor). Control tubes contain labeled antigen and either buffer or antiserum. After incubation for 3 hr at 37°C, chilling to 4°C, and centrifugation at 4°C at 10,000 × *g* for

[1] These tumors have also been successfully passaged at Stanford in (NZB × BALB/cGa)F$_1$.

15 min, 50 μl samples of the supernatant are removed and counted in a well type crystal scintillation counter. The per cent of inhibition is then determined from the following formula:

Per cent inhibition = (1 − I/C) 100 where I = per cent precipitation of labeled antigen in the tube containing labeled antigen, antiserum, and inhibitor; and C = per cent precipitation of labeled antigen in the control tube containing labeled antigen and antiserum.

Protein Conjugation to Polyaminopolystyrene (PAPS).—Conjugation of myeloma proteins to PAPS was performed by the diazo coupling method of Webb and LaPresle (27). The weight ratio of PAPS to protein was approximately 10:1, and the reaction was carried out at pH 8.2. Unreacted diazonium groups were blocked with glycine. Absorption of serum was performed by passage through a column of PAPS-protein conjugate.

RESULTS

Antigenic Specificities on Normal γG_{2a}-Immunoglobulins.—In this further analysis of the Ig-1 isoantigenic specificities on the class γG_{2a} the same methods of determination and rules for defining specificities have been followed as previously described (6).

In listing the data used to define the presence or absence of each specificity in each of the type strains, the following notation is used: An I^{125}-labeled preparation of gamma globulin is indicated with an asterisk following the symbol of the strain from which it was prepared, (for example, C3H*), while normal sera used in inhibition assays are listed by the strain symbols (e.g., C3H). The symbols C3H*-C57BL anti-C3H refer to the use of a labeled C3H gamma globulin preparation with a C57BL anti-C3H antiserum in an inhibition assay. The statement, "(C3H 8)" means C3H has specificity 8. The statement, "(C57BL − 8)" means C57BL does not have specificity 8. Myeloma proteins have occasionally been substituted for preparations of normal gamma globulin, when the myelomas have been found to carry all the isospecificities present on the corresponding class of gamma globulin from normal serum. Thus, in paragraph 5 under γG_{2a}-immunoglobulin RPC-5 has been used in place of C3H gamma globulin.

The Ig-1 isoantigenic specificities of γG_{2a} defined by the available antisera for the type strains are listed in Table I.

1. Specificity 8 was previously defined by the failure of SEA (until then undifferentiated from C3H) to completely inhibit C3H*-C57BL anti-C3H precipitation. The type strain distribution was (C3H 8; C57BL −8; A/J 8; SEA −8) (6).

2. A failure of the other type strains to completely inhibit this same reaction cannot however be used to determine whether they have specificity 8 or not, since these strains lack at least one other specificity detected in this reaction. Therefore, the same C57BL anti-C3H antiserum was used with the iodinated gamma globulin of each of the other type strains to see whether that reaction could be completely inhibited by C3H but not by SEA. Such a finding, could only be interpreted as the presence of specificity 8 in the labeled antigen. It was found that the precipitation of DBA* AKR*, and RIII* by C57BL anti-C3H is completely inhibited by both C3H and SEA. CE*-C57BL anti-C3H precipitation is not completely inhibited by SEA. Therefore CE is the only one of these 4 type strains to have specificity 8 (DBA −8; AKR −8; CE 8; RIII −8).

3. NZB anti-NZC precipitates C3H* (NZC has the same allele, Ig-1^a, as C3H;

and NZB has the same allele Ig-1^e as A/J). Therefore C3H has at least one new specificity not previously described (C3H 9; A/J −9).

4. C57BL*-NZB anti-NZC, and CE*-NZB anti-NZC precipitations are both completely inhibited by all type strains except AKR and A/J which do not inhibit. Therefore all 6 of these strains share at least 1 specificity detected by this antiserum, (C57BL 9; DBA 9; AKR −9; CE 9; RIII 9; SEA 9).

5. RPC-5*-NZB anti-NZC precipitation is completely inhibited by C3H and SEA, is not inhibited by AKR or A/J; and is partially inhibited by C57BL, DBA, CE, and RIII. Therefore in addition to detecting 9, this reaction detects a new specificity, 10, (C3H 10; AKR −10; A/J −10; SEA 10).

TABLE I

Antigenic Specificities Controlled by Ig-1 Alleles on γG_{2a}-Immunoglobulins

Allele	Type strain*	Antigenic specificities									
		1	2	3	4	5	6	7	8	9	10
Ig-1^a	BALB/c‡J	1§	2	—¶	—	—	6	7	8	9	10
Ig-1^b	C57BL/10J	—	—	—	4	—	—	7	—	9	—
Ig-1^c	DBA/2J	—	2	3	—	—	—	7	—	9	—
Ig-1^d	AKR/J	1	2	—	—	5	—	7	—	—	—
Ig-1^e	A/J	1	2	—	—	5	6	7	8	—	—
Ig-1^f	CE/J	1	2	—	—	—	—	—	8	9	—
Ig-1^g	RIII/J	—	2	3	—	—	—	—	—	9	—
Ig-1^h	SEA/Gn	1	2	—	—	—	6	7	—	9	10

* Other strains referred to in this paper carry the following alleles: 129/J, C3H/HeJ, NZC/Bl,-Ig-1^a; LP/J,-Ig-1^b, NZB,-Ig-1^e.

‡ We have changed the Ig-1^a type strain from C3H/HeJ to BALB/cJ because of the availability of BALB/c plasma cell tumors.

¶ Dash indicates specificity is absent.

§ Number indicates specificity is present.

Antigenic Specificities on Normal γG_{2b}-Immunoglobulins.—Many isoantisera have been tested for their ability to precipitate 2 I^{125}-labeled γG_{2b}-myelomas. Since no method for separating γG_{2b}- from γG_{2a}-immunoglobulins of normal mouse serum has so far been found, we are restricted in the analysis of γG_{2b} antigenic specificities to the use of the γG_{2b}-myelomas. Hence inhibition assays have been performed with them in the manner described for the γG_{2a}-isoantigens, but using γG_{2b}-myeloma proteins as labeled antigens. These assays are not inhibited by γG_{2a}-myeloma proteins tested. MPC-11 and MPC-31 are BALB/c myelomas, GPC-5 is an NZB myeloma (see later).

We have previously described isoantigens of γA-immunoglobulins and termed the responsible locus Ig-2. The isoantigenic locus for γG_{2b}-immunoglobulin is therefore called Ig-3. Some of these antigens have also been described by Lieberman et al. (7).

1. MPC-31*-LP anti-129 precipitation is completely inhibited by C3H, DBA, CE, RIII, and SEA, is partially inhibited by AKR and A/J, and is not inhibited by C57BL. This reaction therefore defines a minimum of 2 specificities, (C3H 1, 2; C57BL −1, -2; DBA 1, 2; AKR 1 or 2; A/J 1, −2; CE 1, 2; RIII 1, 2; SEA 1, 2).

2. GPC-5*-BALB/c anti-NZB precipitation is completely inhibited by AKR and A/J and is not inhibited by any other type strains. This reaction therefore defines specificity 3, (C3H −3; C57BL −3; DBA −3; AKR 3; A/J 3; CE −3; RIII −3; SEA −3) (TableII).

Analysis of Isoantigenic Specificities on Myeloma Proteins GPC-5, GPC-7, and GPC-8.—Fifty μl samples of serum from GPC-5, GPC-7, and GPC-8

TABLE II

Antigenic Specificities Controlled by the Ig-3 Locus on γG_{2b}-Immunoglobulins

Type strain*	Antigenic specificities			
	1		2	3
BALB/cGa	1		2	—
C57BL/10J	—		—	—
DBA/2J	1		2	—
AKR/J	1	or	2	3
A/J	1		—	3
CE/J	1		2	—
RIII/J	1		2	—
SEA/Gn	1		2	—

* The strains listed are the type strains for Ig-1.

tumor-bearing mice were separated by electrophoresis as described in methods. The myeloma protein containing fractions were labeled with I^{125}, and the precipitability of each of the 3 was determined with several mouse isoantisera (Table III). These results allow some specificities to be assigned and others to be inferred. All specificities not listed were absent; i.e.,

GPC-5: Ig-1.5 and/or Ig-3.3

GPC-7: Ig-1.1,2,?8; Ig-1.6 and/or Ig-1.7; Ig-1.5 and/or Ig-3.3

GPC-8: Ig-1.1,2,?8; Ig-1.6 and/or Ig-1.7; Ig-1.9 and/or Ig-1.10

Several specificities cannot be assigned solely on the basis of precipitation tests. For example, Ig-1.6 and Ig-1.7 are both detected by CE anti-129, which could precipitate GPC-7 and GPC-8 through the presence of either or both of these specificities on the myeloma protein. To distinguish between these possibilities inhibition tests were performed.

1. GPC-7*-CE anti-129 precipitation is completely inhibited by C3H, and only partially by C57BL. This indicates that both Ig-1.6 and Ig-1.7 are on GPC-7, (GPC-7, Ig-1.6,7).

2. GPC-7*-CE anti-129 was completely inhibited with 0.007 μl of GPC-8 whole serum whereas 0.1 μl of several NZB F_1 hybrid sera was needed to completely inhibit. This indicates that GPC-8 myeloma protein carries specificities Ig-1.6 and Ig-1.7, as these were previously shown to be detected in this assay, (GPC-8, Ig-1.6,7).

3. Both GPC-7*-C57BL anti-C3H and GPC-8*-C57BL anti-C3H precipitations are completely inhibited by C3H but only partially inhibited by SEA. This shows that both myelomas carry Ig-1.8, (GPC-7 and GPC-8, Ig-1.8).

4. GPC-8*-NZB anti-NZC precipitation is completely inhibited by C3H but only partially inhibited by C57BL. This shows that GPC-8 carries both Ig-1.9 and Ig-1.10, (GPC-8, Ig-1.9,10).

TABLE III

Precipitation of I^{125}-Labeled Myeloma Proteins by Various Isoantisera

Antiserum	Antigenic specificities detectable by antiserum		Precipitation of I^{125}-labeled myeloma*		
	Ig-1	Ig-3	GPC-5	GPC-7	GPC-8
C57BL anti-C3H	1, 2, 6, 8	—	−	+	+
C3H anti-DBA/2	3	—	−	−	−
C57BL anti-DBA/2	2, 3	—	−	+	+
BALB/c anti-C57BL	4	—	−	−	−
BALB/c anti-NZB	5	3	+	+	−
RIII anti-129	1	—	−	+	+
NZB anti-NZC	9, 10	—	−	−	+
CE anti-129	6, 7	—	−	+	+
LP anti-129‡	—	1, 2	−	−	−

* −, represents less than 2% precipitation; +, represents greater than 50% precipitation (usually between 70% to 90%).

‡ Absorbed with RPC-5 myeloma conjugated to polyaminopolystyrene resin.

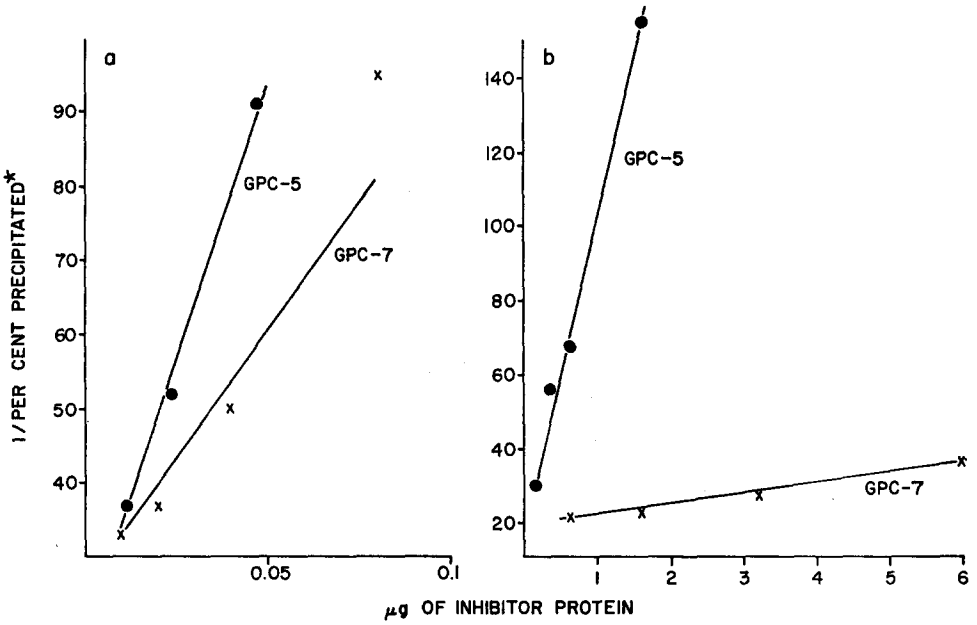
The remaining specificities to be assigned are Ig-1.5 and Ig-3.3, which are both defined by the BALB/c anti-NZB serum. The results of inhibition assays using serial dilutions of the myeloma proteins are presented in Fig. 1.

5. GPC-5*-BALB/c anti-NZB precipitation is not completely inhibited by GPC-7. GPC-7*-BALB/c anti-NZB precipitation *is* completely inhibited by similar amounts of either GPC-5 or GPC-7. This indicates that GPC-5 carries Ig-1.5 and Ig-3.3 only one of which is on GPC-7, (GPC-5, Ig-1.5, Ig-3.3).

6. Since GPC-7* is at least 80% precipitable by both BALB/c anti-NZB and RIII anti-129 (anti-Ig-1.1), most (if not all) GPC-7 molecules must carry both Ig-1.1 and the specificity common to GPC-5 and GPC-7. Since Ig-1.1 is a γG_{2a}-specificity (carried on the γG_{2a}-myelomas 5563 and RPC-5), the common specificity *must be* Ig-1.5. The second specificity detected by BALB/c anti-NZB present only on GPC-5, is Ig-3.3.

The complete list of specificities present on the 3 myelomas is given in Table IV. Consistent with the failure of GPC-7 to completely inhibit GPC-5*-BALB/c anti-NZB precipitation, is the finding that absorption of the BALB/c anti-NZB

serum with GPC-7 myeloma protein bound to PAPS still leaves antibodies which react with approximately 95% of GPC-5*.



FIGS. 1 *a* and 1 *b*. Fig. 1 *a*. Inhibition of precipitation of I^{125}-labeled GPC-7 myeloma protein by GPC-5 and GPC-7 myeloma proteins. Antiserum used was BALB/c anti-NZB. Fig. 1 *b*. As for Fig. 1 *a* but using I^{125}-labeled GPC-5 myeloma protein.

TABLE IV

Antigenic Specificities Present on Myelomas Originating in (NZB × BALB/c) F_1 Hybrids

Myeloma	Ig-1 specificities										Ig-3 specificities		
	1	2	3	4	5	6	7	8	9	10	1	2	3
GPC-5	—	—	—	—	5	—	—	—	—	—	—	—	3
GPC-7	1	2	—	—	5	6	7	8	—	—	—	—	—
GPC-8	1	2	—	—	—	6	7	8	9	10	—	—	—
BALB/c normal γG_2	1	2	—	—	—	6	7	8	9	10	1	2	—
NZB normal γG_2	1	2	—	—	5	6	7	8	—	—	1	—	3

Analysis of GPC-5 with Rabbit Antisera.—GPC-5 has previously been typed as a γG_{2b}-myeloma (14). Since it carries an Ig-1 and an Ig-3 specificity further investigation of GPC-5 with rabbit antisera was made. A rabbit antiserum was prepared against purified MPC-11 myeloma protein (a γG_{2b}-myeloma). This antiserum was then absorbed once with 5563 and once with

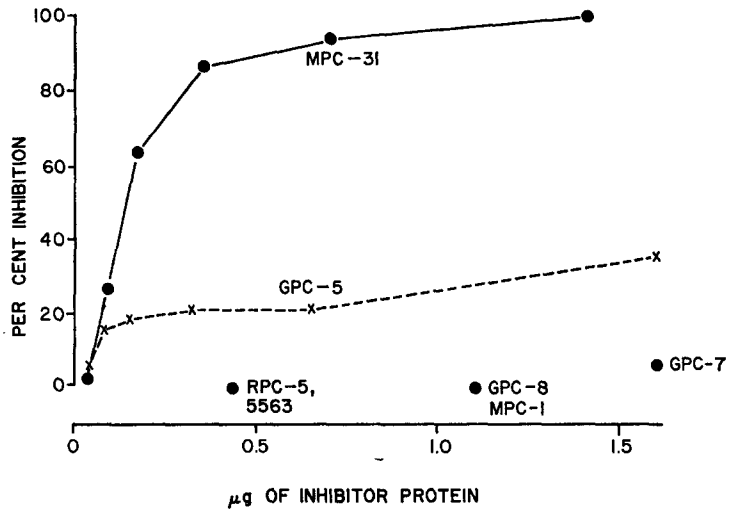


FIG. 2. Inhibition of precipitation of I^{125}-labeled MPC-31 myeloma protein by various purified myeloma proteins. Each tube contains constant amounts of labeled MPC-31 protein and of rabbit anti-MPC-11 myeloma protein serum absorbed separately with 5563 and RPC-5 proteins bound to PAPS. The amount of myeloma protein used to inhibit is given.

TABLE V

Inhibition of Precipitation of I^{125}-Labeled MPC-31 Myeloma by Normal Sera and Myeloma Proteins

Inhibitor	Amount	Inhibition
		%
BALB/c whole serum	0.03 μl	50
NZB " "	1.00 "	0
MPC-31 myeloma	0.02 μg	50
GPC-5 "	0.02 "	0
GPC-5 "	2.00 "	50
GPC-7 "	3.20 "	50

Antiserum: Rabbit anti-MPC-11 myeloma absorbed with A/J normal serum conjugated to PAPS.

RPC-5 protein bound to PAPS (both γG_{2a}-myelomas) to produce a specific anti-γG_{2b}-immunoglobulin antiserum. An inhibition assay was then performed with this antiserum and another I^{125} γG_{2b}-myeloma (MPC-31), to avoid any possible detection of an MPC-11 myeloma specific antigen. The results presented in Fig. 2, indicate that this assay is not inhibited by any γG_{2a}-myeloma (5563, RPC-5, GPC-7, GPC-8), nor by a γA-myeloma (MPC-1). It is com-

pletely inhibited by MPC-31, and is partially inhibited by GPC-5. This partial inhibition confirms that GPC-5 carries γG_{2b}-determinants, but also indicates that it lacks some determinant(s) present on MPC-31. A/J and NZB normal serum were also shown to only partially inhibit this reaction.

Another sample of the rabbit anti-MPC-11 serum was absorbed with A/J normal serum bound to PAPS. This absorbed antiserum still precipitates labeled MPC-31 (completeness of absorption shown by its failure to precipitate I^{125}-labeled A/J gamma globulin). This reaction was inhibited by BALB/c normal serum but not by A/J or NZB normal sera. This indicates the presence of an antigenic determinant on γG_{2b}-immunoglobulins of BALB/c type not present in NZB immunoglobulins. Whereas 0.02 μg of MPC-31 protein gave a 50% inhibition of this assay, 0.02 μg of GPC-5 failed to inhibit at all, 100-fold more GPC-5 protein being needed to give the same degree of inhibition (Table V). A 1% contamination of this fraction with normal BALB/c γG_{2b}-immunoglobulin would account for the inhibition observed with the GPC-5 fraction. This degree of contamination is expectable since this fraction was isolated from the serum of an (NZB $\times$ BALB/c)F_1 mouse carrying GPC-5.

DISCUSSION

The Ig-1 locus of mice, previously shown to be highly polymorphic with 8 alleles detected in some 70 inbred strains, controls isoantigens (allotypes) on γG_{2a}-immunoglobulins. The isoantigens determined by these alleles are comprised of multiple antigenic specificities inherited as a phenogroup. Eight such antigenic specificities had been defined (6). This number has now been increased to 10, and an analysis of the Ig-3 locus, controlling isoantigens on the γG_{2b}-immunoglobulins has revealed at least 3 antigenic specificities.

Before discussing the analysis of the myeloma proteins, some explanation for considering the isoantigens on γG_{2a} and γG_{2b} to be controlled by two distinct loci Ig-1 and Ig-3 may be helpful. The explanation is identical with that previously made in designating a distinct locus, Ig-2, for the allotypes (isoantigens) found on γA-globulins (5). A gene locus is defined as the length of DNA which codes for a species of polypeptide chain. In each individual mouse there are molecules of 5 immunoglobulin classes γG_{2a}, γG_{2b}, γG_1, γA, and γM which are distinguished from one another by at least the Fc fragments of their heavy (H) polypeptide chains (15, 16, 28).

These classes all differ for some of the following properties: molecular size, average electrophoretic mobility (15, 16), physiological (29) and metabolic behavior (30), and in antigens (15, 16). The distinction between γG_{2a} and γG_{2b} was originally made only on the basis of antigenic differences. Subsequently a difference in the physiological activities of γG_{2a} and γG_{2b} was found (31). These differences can all be ascribed to the H-chain found in each class.

Recently, mapping of the tryptic peptides of the Fc fragments (and of the entire H-chains) has shown that γG_{2a}- and γG_{2b}-class distinctions are associated with polypeptide sequence differences. The Fc fragments of all γG_{2a}-myeloma proteins tested gave tryptic peptide fingerprints identical to one another. The Fc fragments of all γG_{2b}-myeloma proteins tested, also gave fingerprints identical with each other, but, the γG_{2a}- and γG_{2b}-patterns were distinctly different in the position of some 18 peptides. In addition some 12 peptides of the 2 types of patterns had the same position (32). This is a very welcome support for the immunochemical findings of cross-reactions and differences of the γG_{2a}- and γG_{2b}-Fc fragments. The γA-fingerprints had previously been shown to be distinct (33).

The above is ample reason for concluding that the molecules belonging to each immunoglobulin class, even within one individual mouse, have a common Fc polypeptide which differs from that of other classes. Thus by the definition of locus (above) we must assign different loci to each immunoglobulin class.

Genetic polymorphism has so far been found only for γG_{2a}, γA, and γG_{2b}. The locus designations we have assigned are Ig-1, Ig-2, and Ig-3 respectively. Ig-1 and Ig-2 have been shown to be closely linked genetically as have Ig-1 and Ig-3 (5, 7). Therefore, these loci are clustered in a chromosome region (in the same sense as that used for the H-2 histocompatibility region (34) which may be called the Ig region. Each locus has multiple alleles coding for the H-chains (or Fc fragments) of the respective immunoglobulins. The concept of several linked loci for mouse immunoglobulin classes is an exact parallel of the postulated multiple loci controlling human γG-H-chain subgroups. As in the mouse, individual genetic factors (Gm specificities) are each associated with a particular H-chain subgroup (21).

Results of analysis of 3 plasma cell tumors induced by paraffin oil injection into (NZB $\times$ BALB/c)F_1 hybrid mice which produce γG_2-myeloma proteins have been presented. NZB and BALB/c carry different alleles at both the Ig-1 and Ig-3 loci thus providing the opportunity to analyze the role of each of the 4 alleles present in the hybrids in determining the allotypes of the myeloma proteins. The two tumors GPC-7 and GPC-8 produce γG_{2a}-myeloma proteins which will be discussed first. BALB/c and NZB carry respectively Ig-1^a and Ig-1^e alleles. The antigens determined by these alleles have several antigenic specificities in common, i.e. 1, 2, 6, 7, and 8, and each has at least one specificity not present in the other; i.e., BALB/c has 9, 10; and NZB has 5 (Table I). Both GPC-7 and GPC-8 have all the specificities shared by BALB/c and NZB, but GPC-7 has specificity 5 (associated with the Ig-1^e allele), and does not have 9 and 10, while GPC-8 has the specificities 9 and 10 (associated with the Ig-1^a allele), and does not have 5. Thus, each of these two γG_{2a}-myeloma proteins is carrying the antigenic specificities determined by only *one or the other* of the parental Ig-1 alleles present in the mouse in which the tumor arose.

Individual myeloma proteins in man of the We and Vi class (21) also called γ_{2b} and γ_{2c} (35) have (with one exception) (24) been found to have only one of the allelically controlled antigens such as Gma, b, or f (20–22, 24), even when the normal gamma globulin population is carrying several of these antigens. In rabbits heterozygous at the Ab locus (36), and in mice heterozygous at the Ig-1 locus (37), the two allelically controlled isoantigens have been shown to be located on different molecules. It has not however been conclusively eliminated that a small proportion (say 5%), of "heterozygous" molecules also exist, and indeed such molecules can be produced in vitro by hybridization (38). Rabbits heterozygous at the Ab locus have been examined by Pernis et al. (39) who found that the 2 allelic types of protein were always synthesized in different cells. Thus the results with GPC-7 and GPC-8 are in agreement with the general observation that both normal and malignant cells express only one H-chain or L-chain allele (at least at the time of examination).

A further point should be emphasized about these 2 myelomas. In both cases, the protein studied has been isolated from the original host of the tumor. It has been shown that in some mice which have been treated with prolonged injections of adjuvants, and in whom plasma cell tumors arise, separate tumors, i.e. producing different immunoglobulin polypeptide chains, can be found in the one host (40). It might be possible then that in the GPC-7 and GPC-8 lines, there is in fact more than one discrete tumor and that the antigenic specificities are those of several myelomas. This possibility is eliminated by the finding that various unispecific antisera precipitate up to 90% of the myeloma protein (allowing a maximum of 8% contamination by total normal γG_2-globulins). These antigenic specificities are hence all on the same molecules.

The third myeloma studied, GPC-5, may be of particular value in understanding at least one aspect of the genetic control of immunoglobulins. This myeloma, which is also derived in a (NZB $\times$ BALB/c)F_1 hybrid has the antigenic specificities of only 1 parental strain (NZB). It types unambiguously as γG_{2b}- myeloma with 2 rabbit antisera specific for γG_{2b}. Results presented in this paper demonstrate that one of these rabbit antisera has antibody activity towards specificities on BALB/c γG_{2b} not present on NZB γG_{2b} and adds further confirmation of GPC-5 being of NZB γG_{2b}-type. The only antigenic specificities found on GPC-5 are both defined by the BALB/c anti-NZB serum. This myeloma carries only 1 of the 2 Ig-3 specificities of normal NZB γG_{2b}, namely Ig-3.3, but even more remarkable is the finding that it also carries a specificity which is found on normal and myeloma NZB γG_{2a}-immunoglobulins, namely Ig-1.5. On the basis of inhibition data for Ig-1.5, and with direct testing by specific antiserum to Ig-3.3, at least 95% of molecules of GPC-5 carry both specificities.

GPC-5 is therefore the first analyzed mouse myeloma protein *not* carrying the entire set of isospecificities normal to its class and genotype, that is, all

γG_{2a}- or γG_{2b}-molecules previously analyzed do carry all of the antigenic specificities determined by the respective allele present in that individual. GPC-5 is also the first example of a mouse myeloma protein carrying antigenic specificities, and inferentially the amino acid sequences of 2 classes of immunoglobulins.

To account for the occurrence of Ig-1.5 and Ig-3.3, in the same molecules of GPC-5, two basic alternative explanations might be proposed:

1. That this type of molecule, despite lack of prior detection, is a normal γG_2-component synthesized by a small proportion of plasma cells prior to tumor induction;

2. That this molecular species did not exist prior to tumor induction. Detailed comments on the specific mechanism of origin of GPC-5 should at present be limited to a consideration of when the recombinational or mutational event occurred. In the case of alternative 1, immediately preceding, this would have been either in the germ line or in somatic cell division of these mice. Alternative 2, immediately preceding, would require that this event was directly related to the actual tumor induction.

These results provide great stimulation to continue the isoantigenic analysis of myeloma proteins, in the expectation that they will throw further light on the genetic control of immunoglobulin structure.

SUMMARY

Further analysis of the isoantigens (allotypes) of 2 classes of normal mouse immunoglobulins, γG_{2a} and γG_{2b}, has shown a minimum of 10 specificities for the Ig-1 locus (controlling γG_{2a}-antigens) and 3 specificities for the Ig-3 locus (controlling γG_{2b}-antigens).

Three γG_2-myeloma proteins of plasma cell tumors induced in (NZB $\times$ BALB/c)F_1 mice have been analyzed for the isoantigens they carry. NZB mice are genotypically Ig-1^e Ig-3^e, while BALB/c are Ig-1^a Ig-3^a. Two of the myeloma proteins are γG_{2a}-globulins. One of these, GPC-7, carries all the isoantigenic specificities of the Ig-1^e allele while the other, GPC-8, carries all the isoantigenic specificities of the Ig-1^a allele. Thus only one of the parental alleles of the mouse in which the tumor arose is expressed in each of these myeloma proteins.

The third myeloma protein GPC-5, also carries the antigens of only one parental strain (NZB). However GPC-5, a γG_{2b}-globulin, carries only one of the Ig-3 specificities normally associated with γG_{2b}-globulins of NZB. Most remarkably it also carries one Ig-1 specificity normally associated with γG_{2a}-globulins of NZB. This is the first analyzed mouse myeloma shown (*a*) to express some but not all the antigenic specificities normally associated with an allele and (*b*) to carry antigenic specificities controlled by two distinct immunoglobulin loci. The implications of these findings are discussed in relation to the genetic control of immunoglobulins.

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