GENETICS OF A NEW IgV_H (T15 IDIOTYPE) MARKER IN THE MOUSE REGULATING NATURAL ANTIBODY TO PHOSPHORYLCHOLINE

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(Received for publication 15 January 1974)

The formal genetics of immunoglobulins has been developed primarily through the use of markers that have been assigned to the constant parts of the immunoglobulin molecules (IgC_L and IgC_H). However, the functional antigen-binding parts of the immunoglobulins are located on the variable-region (V-region)[1] segments of the molecule and are controlled by a much larger number of IgV_L and IgV_H genes. Genetic analysis of V-region related functions is complicated by the vast numbers of different types of immunoglobulin molecules. Progress, however, has been made in this area by the use of homogeneous antibodies (1–5) or myeloma proteins that have known hapten-binding specificities (6–10). Here is has been possible to retrieve a specific functional group of immunoglobulins by their ability to bind the specific antigen. Further, the availability of homogeneous immunoglobulins in sufficient quantity has permitted both structural and antigenic studies. Homogeneous immunoglobulins have individual antigenic specificities that are located on the variable parts of the molecule which can be used to distinguish one species of immunoglobulin from all the others. These markers, called individual antigenic specificities (11) or idiotypes (12), are useful in genetic studies when specific immunoglobulin molecules carrying these idiotypes can be elicited in the organism by immunization (4, 5, 13–16) or are found normally in serum as a natural antibody.

Three idiotypic systems each related to different haptens have been described in the mouse: (*a*) the λ-type immunoglobulins with $\alpha 1 \rightarrow 3$ dextran-binding activity that share cross-reacting idiotypic determinants with the $\alpha 1 \rightarrow 3$ dextran-binding J558 myeloma protein (15, 16), (*b*) the antiarsonate antibodies elicited in strains A/He and AL/N that share a cross-reacting idiotypic specificity (13, 14), and (*c*) the homogeneous Group A antistreptococcal carbohydrate antibodies that carry the A5A idiotype (5, 17). In addition, Sher and Cohn (18) have described the genetics of a phosphorylcholine antibody in the mouse. In a previous study we showed that the phosphorylcholine-binding myeloma proteins, produced by five independently induced plasmocytomas

[1] *Abbreviations used in this paper:* HA, passive hemagglutination; HI, hemagglutination inhibition; hi, high; lo, low; PnC, pneumococcus C polysaccharide; SRBC, sheep red blood cell; RI, recombinant inbred; V-region, variable region B6, C57BL/6; C, BALB/c.

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in BALB/c mice (the S63, S107 plasmocytomas from the Salk Institute, and the HOPC8 [H8], TEPC15 [T15], and MOPC299 plasmocytomas from the National Institutes of Health), shared the same idiotypic determinant (9) called here the T15 idiotype for brevity. Subsequently Sher et al. (19) found three more similar proteins. Cosenza and Köhler (20, 21) demonstrated that normal cells in the immunized BALB/c mouse produced antiphosphorylcholine antibodies with the T15 idiotype.

In the present study we describe the genetics of a naturally occurring phosphorylcholine-binding antibody associated with the T15 idiotype. The strain distribution of this marker (T15) indicates that it is not the same as the one described by Sher and Cohn (18). Further, we have been able to show conclusively that the T15 idiotype marker is linked to the IgC_H-locus of the BALB/c mouse.

Materials and Methods

Myeloma Proteins.—The IgA myeloma proteins used in this study, TEPC 15 (T15), HOPC 8 (H8) MOPC 167 (M167), and McPC 603 (M603), have been previously described (9, 22). These proteins, produced by four plasmacytomas of independent origin in strain BALB/c mice, were all shown to bind phosphorylcholine (8). Two of the proteins, T15 and H8, were found to share the same idiotype, while the others had their own unique individual antigenic specificities (9). The myeloma proteins used in the experiments presented here were isolated by immunoadsorption using the methods of Chesebro and Metzger (23).

Anti-Idiotype Antisera.—Anti-T15 antisera were prepared in A/He mice by immunization with T15 isolated by ammonium sulfate precipitation. A/He anti-H8 antisera were produced in the same strain by immunization with the reduced and alkylated monomeric form of H8 that had been isolated by immunoadsorption (23). None of the antisera used reacted to IgA allotypes.

Anti-R36 Pneumococcus Antisera.—Some strains of mice were immunized with the R36 strain of pneumococcus kindly supplied to us by Dr. Alex Tomasz, The Rockefeller University, New York. A single i.p. injection of 0.5×10^9 organisms was given each mouse and the serum was tested 1 wk later for inhibition of anti-T15 idiotype.

Passive Hemagglutination (HA) and Hemagglutination Inhibition (HI) Assays for Detection of T15 or H8 Idioptye.—Details of the HA and HI methods using the microtiter system have been previously described (24). H8 or T15 proteins purified by immunoadsorption were coupled to sheep red blood cells (SRBC) by the chromic chloride method (25).

Before use, all antisera were absorbed with SRBC before determining the HA titer to H8 or T15 coupled SRBC. The HI titer was determined by utilizing the dilution of antiserum two tubes from the endpoint of the HA titer and using as inhibitors myeloma proteins purified by immunoadsorption, normal serum, or immune serum. All inhibitors were preabsorbed with SRBC before use in the HI tests. In a few instances the inhibitors (normal and immune sera) were absorbed with Sepharose phosphorylcholine beads or a suspension of washed 10^9 killed pneumococci (R36 strain).

Mouse Strains.—Most of the inbred strains of mice used in the present study were obtained from various laboratories at the National Institutes of Health. In particular, BALB/c mice and C57BL/Ka mice were obtained from different mouse colonies. The Bailey recombinant inbred (RI) strains C × BD, C × BE, C × BG, C × BH, C × BI, C × BJ, and C × BK (26) were bred at the National Institutes of Health or were kindly supplied by Dr. Donald W. Bailey, Jackson Laboratories, Bar Harbor, Maine. These seven strains were derived from seven different pairs of (C57BL/6 × BALB/c)F_2 mice (26). The congenic CB20 mice were

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derived from an introgressive cross (27) in which the C57BL/Ka IgC_H group was introduced onto the BALB/c background by 20 consecutive backcrosses. This was accomplished by using (C57BL/Ka × BALB/c)F_1 mice which were mated to BALB/c and the backcross progeny carrying the unassigned "2" allotypic determinant were selected and backcrossed to BALB/c. This same process was repeated 20 times. 20th backcross progeny with the unassigned 2 allotypic determinant were then mated to each other, and mice homozygous for the unassigned 2 allotypic determinant were selected as parents for the new strain CB20 which has since been maintained by continuous brother-sister mating. To check the homozygosity of the CB20 strain, the CB20 were mated to DBA/2. All the progeny from this cross possessed the allotypic determinants of the CB20 (unassigned 2 determinant) and the DBA/2 (G^3 allotype) and none possessed the allotypic determinants of BALB/c. This confirmed the homozygosity of the CB20 strains.

BAB-14 mice were developed by Dr. Leonard Herzenberg, Stanford University, from the 14th backcross stock of our CB introgressive cross. These mice were made homozygous for the C57BL/Ka IgC_H allotype by mating the 14th backcross progeny together. They have been maintained by brother-sister mating ever since. Sera from BAB-14 mice were kindly provided by Dr. Martin Weigert, The Salk Institute, La Jolla, Calif.

RESULTS

Specificity and Sensitivity of the Anti-T15 Idiotype Antibody—The antisera used in the present study were raised in strain A/He mice immunized with TEPC15 (T15) HOPC8 (H8) and McPC603 (M603) myeloma proteins of BALB/c origin. These antisera were tested for hemagglutination of SRBC that were coated by the chromic chloride method with highly purified (immunoadsorbed) phosphorylcholine-binding IgA myeloma proteins T15, H8, M603, and M167. As may be seen in Table I the antisera were highly specific for the immunizing antigen. The titer of anti-T15 and H8 antisera were very high, ranging from 1/16,000 to 1/32,000. These antisera lack allotypic activity as demonstrated by their failure to react with the M167 and M603 IgA myeloma protein-coated SRBC. In fact, in numerous attempts, we have never succeeded in producing antiallotype antisera to T15 or H8 myeloma proteins in A/He mice, whereas with M603 and M167 we are uniformly successful. All of these proteins (T15, H8, M603, and M167) exhibited the $A^{12, 13, 14}$ IgC_H determinants when tested with an antiallotype antiserum prepared with another BALB/c IgA myeloma protein. The anti-M603 antiserum that was used in this

TABLE I

Specificity and sensitivity of Anti-T15 and Anti-H8 Antibody

Antiserum		HA titer for SRBC coupled with:			
Specificity	No.	T15*	H8	M603	M167
Anti-T15	6994	1/16,000	1/16,000	0	0
Anti-H8	7641	1/32,000	1/32,000	0	0
Anti-M603	6792	0	0	1/64,000	0

* TEPC 15 (T15), HOPC 8 (H8), McPC 603 (M603), and MOPC 167 (M167) are BALB/c IgA myeloma proteins binding phosphorylcholine.

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study was selected for its very high idiotype and very low allotype HA titer. The reactivity of the antiallotype antibodies in this antiserum was easily removed by absorption with an unrelated BALB/c IgA myeloma protein which made the antiserum highly specific for the idiotypic determinants alone. Also, the antisera to H8 and T15 agglutinated both H8- and T15-coated SRBC to the same titer, confirming with the sensitive hemagglutination system the observation previously described using the Ouchterlony method, that H8 and T15 share the same idiotypes.

Quantitation of the T15 and H8 idiotype was made using the HI method. An antiserum dilution that was four times more concentrated than the limiting hemagglutination dilution was first determined. Hemagglutination inhibition of preparations containing known amounts of T15 and H8 proteins was determined. Two different immunoadsorbed preparations of H8 and T15 were brought to equal concentrations by optical density and tested as inhibitors in five different systems. As may be seen (Table II) the four preparations were roughly similar in HI titer within each of the four systems where either H8 or T15 was used as the antigen. None of the preparations inhibited an anti-M603-M603 system. The variations in log 2 titer between systems was probably a function of the potency of the antiserum dilution.

A pepsin Fab fragment from the T15 protein was prepared from immunoadsorbed T15 protein and then further purified on Sephadex G100. This preparation inhibited the 6994 anti-T15-T15 systems as efficiently as the IgA monomer preparation, thus demonstrating the location of the idiotypic determinant on the pepsin Fab fragment.

The specificity of the system was further demonstrated by showing that 13 other purified myeloma proteins failed to inhibit the anti-T15-T15 system; these included the two γA phosphorylcholine-binding myeloma proteins M603 and M167, three γG proteins, one γH protein, and six other γA proteins (Table II).

A surprising finding was that normal BALB/c serum from six different mice inhibited the four highly specific systems (anti-T15-T15, anti-T15-H8, anti-H8-H8, and anti-H8-T15). The amount of protein containing the T15 idiotype in normal BALB/c serum was estimated from these results to range from 8 to 64 μg/ml.

T15 Idiotype in Normal Serum of Germ-Free and Conventionalized BALB/c Mice.—The sera from two separate groups of germ-free mice, each derived from several litters, were examined for the presence of the T15 idiotype (Table III). These sera were very kindly provided by Dr. Richard Asofsky, NIAID. The T15 idiotype was not found in the sera of the 40 germ-free BALB/c mice tested. The mice were then conventionalized by placing them in a normal environment and again their sera were tested for the T15 idiotype at different times following conventionalization. The T15 idiotype was found as early as 5 days following conventionalization and only 3 of 37 mice failed to show some levels of T15

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TABLE II

Inhibition of Hemagglutination of H8, T15, and M603 Coupled SRBC with Anti-Idiotype Serum by Phosphorylcholine-Binding Myeloma Proteins, Normal Serum, and Other Myeloma Proteins of BALB/c Origin

Inhibitor	Mg/ml in 1st tube	Log 2 hemagglutination inhibition titer				
		H8 Cells		T15 Cells		M603 Cells
		Anti-T15 6994*	Anti-H8 7641*	Anti-T15 6994*	Anti-H8 7641*	Anti-M603 6792*
H8 prep-1‡	0.125	7	5	9	6	0
H8 prep-2‡	"	7	6	7.5	3	0
T15 prep-1‡	"	7	7	11	4.5	0
T15 prep-2‡	"	7.5	7.5	9.5	5.5	0
T15 (pepsin fab)				8.0		
BALB/c 1	NS§	6	5.5	7	5.5	0
" 2	"	6	5	7	5	0
" 3	"	6	3.5	6	5	0
" 4	"	5	3	4	3.5	0
" 5	"	6	5	6.5	4.5	0
" 6	"	4	4.5	4.5	5	0
M167	1.0	0		0		0
M603	1.0	0		0		>12
Others‖	1.0			0		

* The titer of antiserum used was four times the concentration of the HA end point.

‡ Each preparation was prepared separately by immunoadsorption. In this procedure ascites containing the myeloma protein was reduced with 0.01 M dithiothreitol and alkylated with 0.022 M iodoacetamide. This procedure converts the IgA myeloma proteins to monomeric (7S) forms.

§ Normal serum.

‖ Eleven other purified BALB/c myeloma proteins none of which bind phosphorylcholine were tested with T15 cells and anti-T15 antiserum 6994: γG (γ2a) AdjPC5, LPC1, UPC10; γH (γ2b) UPC120, and γA proteins MOPC315, SAPC10, XRPC24, TEPC601, TEPC191, and XRPC44.

idiotype. There appeared to be no sex distinction in the development of the T15 idiotype (Table III). In comparison to the levels of T15 idiotype obtained in normal sera of BALB/c (Tables II and IV), the levels of T15 idiotype obtained in conventionalized mice derived from germ-free conditions were appreciably lower than in normal mice.

T15 Idiotype in Normal Sera of Inbred and Recombinant Inbred Strains of Mice.—Other inbred strains of mice were surveyed for the presence of T15 idiotype in their normal serum (Table IV). Mice from five different immunoglobulin heavy-chain linkage groups (IgC_H) and of different *H-2* types were selected and their normal sera tested for the presence of the T15 idiotype.

High levels of T15 idiotype were present in normal serum of BALB/c, C57L, C58, ST, and 129. Absence or low levels of T15 were detected in CBA, C3H, C57BL/10, SJL, B10.D2, DBA/2, RIII, A, AL, AKR, NZB, and NH.

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TABLE III

Inhibition of Hemagglutination of T15-SRBC with Anti-T15 Idiotypic Antiserum with Serum From Germ-Free and Conventionalized GF BALB/c Mice

	Total no. mice	Sex	Log 2 HI titer						
			0	1	2	3	4	5	6
Germ-Free Group 1	5	♂	5	—	—	—	—	—	—
" "	19	♀	19	—	—	—	—	—	—
Conventionalized—10 days	1	♂	—	—	—	1	—	—	—
" 10 "	4	♀	—	—	—	3	1	—	—
" 16 "	1	♂	—	—	—	—	1	—	—
" 16 "	5	♀	—	—	—	4	1	—	—
" 22 "	1	♂	—	—	1	—	—	—	—
" 22 "	4	♀	1	—	1	2	—	—	—
" 35 "	1	♂	—	—	—	—	1	—	—
" 35 "	4	♀	—	—	—	1	1	2	—
" 56 "	1	♂	—	1	—	—	—	—	—
" 56 "	1	♀	—	—	—	—	—	1	—
Germ-Free Group 2	16	♂	16	—	—	—	—	—	—
Conventionalized 5 days	3	♂	—	—	—	2	—	1	—
" 11 "	5	♂	—	2	2	1	—	—	—
" 18 "	3	♂	—	—	1	—	2	—	—
" 26 "	3	♂	2	1	—	—	—	—	—

So far the T15 idiotype is associated with some but not all the strains in the a^1 IgC_H group and not with strains having the a^2, a^3, a^4, or a^5 IgC_H groups (see footnote in Table IV). There was no association of the *H-2* type with the presence of the T15 idiotype.

Presence of T15 Idiotype in Normal Sera Following Absorption with SRBC.— The HI method used in these studies involves the coating of chromic chloride-treated SRBC with the myeloma protein antigen (T15) to be assayed. Because of the presence of a natural SRBC agglutinin in normal mouse sera of some strains, particularly C57BL, all normal and immune sera used as inhibitors were preabsorbed with SRBC before testing for the presence of T15 idiotype.

A further possibility existed that cross-reacting antigens may exist between SRBC and T15, and this was determined by testing for T15 inhibition in sera before and after absorption with SRBC (Table V).

The natural SRBC agglutinin titer is shown for selected sera from C57BL/6, BALB/c, Bailey's recombinants C × BD and C × BG, and congenic CB20 mice. The highest log 2 HA titer for SRBC agglutinins were found in the C57BL/6 mice and varied from 1–10.

Absorption with SRBC revealed the presence of low levels of T15 idiotype in B6 serum and the absence of T15 in the serum of C × BD and CB20 strains. The high levels of T15 idiotype in C × BG and BALB/c were unaffected by absorption with SRBC and indicated that there was no cross-reaction between SRBC antigens and the T15 idiotype.

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TABLE IV

Presence of T15 Idiotype in Normal Sera of Various Inbred Strains

Strains (inbred)	*H-2*	IgC_H allotype group‡	Total no.	Log 2 HI titer*										
				0	1	2	3	4	5	6	7	8	9	10
BALB/c	*d*	a^1	43	—	—	—	3	6	9	7	6	5	4	3
CBA	*k*	"	23	21	2	—	—	—	—	—	—	—	—	—
C3H	*k*	"	29	25	4	—	—	—	—	—	—	—	—	—
C57L	*b*	"	10	—	—	—	—	2	—	2	4	—	2	—
C58	*k*	"	10	—	—	—	—	—	1	5	1	—	3	—
ST	*k*	"	14	—	—	4	2	1	5	1	1	—	—	—
129	*b*	"	9	—	—	—	—	4	2	3	—	—	—	—
C57BL/10	*b*	a^2	12	12	—	—	—	—	—	—	—	—	—	—
SJL	*e*	"	10	10	—	—	—	—	—	—	—	—	—	—
B10.D2	*d*	"	26	24	—	—	2	—	—	—	—	—	—	—
DBA/2	*d*	a^3	13	13	—	—	—	—	—	—	—	—	—	—
RIII	*r*	"	16	16	—	—	—	—	—	—	—	—	—	—
A	*a*	a^4	8	8	—	—	—	—	—	—	—	—	—	—
AL	*a*	"	8	8	—	—	—	—	—	—	—	—	—	—
AKR	*k*	"	16	16	—	—	—	—	—	—	—	—	—	—
NZB	*d*	"	8	8	—	—	—	—	—	—	—	—	—	—
NH	?	a^5	14	8	4	1	1	—	—	—	—	—	—	—

* All sera were preabsorbed with SRBC.

‡ Characteristic sets of allotype markers located on different heavy chains are found concordantly in different inbred strains (28, 29). $a^1 = G^{1,6,7,8}H^{9,11,22}A^{12,13,14}F^{8,19}$; $a^2 = {}^2G^{-}H^{9,16,22}A^{15}F^{slow}$; $a^3 = G^{3,8}H^{9,11}A^{-}F^{8,19}$; $a^4 = {}^{10}G^{4,6,7,8}H^{4,23}A^{13,17}F^{8,19}$; and $a^5 = G^{5,7,8}H^{9,11}A^{14}F^{8,19}$. The abbreviated symbol a^1, a^2 etc. is a convenient means for designating a group of closely related IgC_H allotypic determinants. Several of these, e.g., a^3 and a^4 groups have been further subdivided (29, 30) by minor antigenic differences; in addition other groups have been found. (27, 31).

Linkage of Genes Controlling the T15 Idiotype on Normal Serum to the IgC_H Locus of BALB/c Mice.—Normal sera of F_2 progeny of BALB/c and C57BL/6, Bailey's RI recombinant inbred strains and congenic CB20 strains were examined for the presence of T15 idiotype (Table VI). Both C57BL/6 and C57BL/Ka sera were tested as these strains were the parental strains used in the Bailey RI strains (C × BD, C × BE, etc) and congenic strains (CB20 and BAB14) respectively. As may be seen in Table VI, only the strains or hybrids that possessed the BALB/c IgC_H locus (a^1) gave high levels of T15 idiotype (log 2 HI titer mean range 5.0–7.4). Sera of C57BL/6 and C57BL/Ka showed low levels of T15 idiotypes (log 2 HI titer mean 0.8 and 1.2, respectively). The (C × B6) F_2 progeny exhibited high levels of T15 idiotype in their sera when they were either homozygous for a^1 allotype (log 2 HI titer mean range 5.7–6.1) or heterozygous for a^1 (log 2 HI titer mean range 5.1–5.5). None of the sera of the homozygous a^2 progeny showed high levels of T15 idiotype (log 2 HI titer mean range 0–0.6).

Among the 36 congenic CB20 mice, including males and females, none of the sera had high levels of T15 idiotype (log 2 HI titer mean range 0.2–2.3). All

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TABLE V

T15 Idiotype in Normal Sera of Various Strains before and after Absorption with SRBC

Strain	Log 2 titer of test serum for:		
	SRBC (HA, preabsorb)	T15-SRBC (HI, preabsorb)	T15-SRBC (HI, postabsorb)
B6	4	0	2
"	10	0	0
"	5	0	3
"	1	0	2
"	7	0	1
C × BD	1	0	0
"	1	0	0
"	2	0	0
"	1	0	0
C × BG	3	6	6
"	0	5	5
"	2	6	6
"	0	8	7
"	0	5	6
C	0	7	7
"	2	9	8
"	2	8	7
"	2	6	6
"	1	8	7
CB20	4	0	0
"	3	0	0
"	1	0	0
"	0	0	0

these strains have the a^2 allotype of the C57BL/Ka. 18 sera from the BAB-14 stock (a congenic strain derived from our CB introgressive cross) also had low titers.

The normal sera of the Bailey RI strains C × BG and C × BJ, which have the a^1 (IgC_H) allotype group of BALB/c, showed high levels of T15 idiotype (log 2 HI titer mean 5.0 and 6.0 respectively). The C × BD, C × BE, C × BH, C × BI, and C × BK which have the C57BL/6 allotype gave low levels of T15 idiotype in their sera (log 2 mean HI titer 0.4, 1.3, 1.6, 0.3, and 1.0 respectively).

Presence of T15 Idiotype in Normal and Immune Sera (Anti-R36 Pneumococcus).—Most of the strains used in the genetic studies to determine linkage of the IgV_H (T15 idiotype) to the BALB/c IgC_H were immunized with R36, a rough strain of pneumococcus having the phosphorylcholine-containing antigen on its surface (Table VII). The sera of these strains in addition to AL were examined for the T15 idiotype before and 1 wk following immunization with the R36 pneumococcus. An increase in the level of T15 idiotype was observed in both female and male BALB/c following immunization (log 2 mean HI

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TABLE VI

Evidence for the Linkage of IgV_H (T15 Idiotype) to IgC_H Locus in BALB/c Mice

Strain	IgC_H allotype group	Total no. mice	HI of T15 idiotype with normal sera (log 2 HI titer)											
			0	1	2	3	4	5	6	7	8	9	10	Mean
Parental strains														
B6	a^2a^2	10	3	1	6	—	—	—	—	—	—	—	—	1.3
B6 ♀	a^2a^2	14	11	—	3	—	—	—	—	—	—	—	—	0.4
BKa	a^2a^2	8	2	2	4	—	—	—	—	—	—	—	—	1.2
C ♀	a^1a^1	10	—	—	—	—	—	—	2	5	1	2	—	7.3
C ♂	a^1a^1	20	—	—	—	1	6	7	4	2	—	—	—	5.1
C	a^1a^1	14	—	—	—	—	3	5	5	1	—	—	—	7.4
F_2 Progeny (C57BL × BALB/c)F_2														
BL ♂	a^2a^2	9	5	3	1	—	—	—	—	—	—	—	—	0.6
BL ♀	a^2a^2	11	11	—	—	—	—	—	—	—	—	—	—	0.0
C ♂	a^1a^1	19	—	—	—	—	3	6	5	4	1	—	—	5.7
C ♀	a^1a^1	19	—	—	—	—	2	2	8	6	1	—	—	6.1
BL×C ♂	a^1a^2	13	—	—	1	—	1	4	4	2	1	—	—	5.5
BL×C ♀	a^1a^2	14	—	—	—	1	3	5	4	1	—	—	—	5.1
Congenic strains (Ig)														
CB20 ♀	a^2a^2	10	—	3	7	—	—	—	—	—	—	—	—	1.7
CB20 ♂	a^2a^2	6	—	—	4	2	—	—	—	—	—	—	—	2.3
CB20 ♂	a^2a^2	20	16	4	—	—	—	—	—	—	—	—	—	0.2
BAB 14	a^2a^2	18	18	—	—	—	—	—	—	—	—	—	—	0.0
Bailey RI strains														
C × BD	a^2a^2	15	11	2	2	—	—	—	—	—	—	—	—	0.4
C × BE	a^2a^2	15	8	—	2	5	—	—	—	—	—	—	—	1.3
C × BG	a^1a^1	15	—	—	—	1	3	5	4	1	1	—	—	5.0
C × BH	a^2a^2	15	5	0	6	4	—	—	—	—	—	—	—	1.6
C × BI	a^2a^2	10	8	1	1	—	—	—	—	—	—	—	—	0.3
C × BJ	a^1a^1	10	1	—	—	—	2	—	3	1	1	1	1	6.0
C × BK	a^2a^2	15	6	3	4	2	—	—	—	—	—	—	—	1.0

titer ♀ 7.8 → 10.6; ♂ 7.4 → 10.1). There was no apparent increase of the low levels of T15 originally present in the normal sera of C57BL/Ka, C × BI, or AL mice following immunization. In the sera of CB20 the titers increased from log 2 mean HI titer of 2.0 → 3.4 for the females, 2.3 → 3.7 for the males, and 0.2 → 1.7 for C × BH.

Absorption of T15 Idiotype in Normal and Immune (Anti-R36) Sera by Sepharose Phosphorylcholine Beads or R36 Pneumococci.—Normal and immune sera (anti-R36) of BALB/c, C57BL/Ka, and CB20 that exhibited some of the higher levels of T15 idiotype that were observed were selected and absorbed with a packed cell suspension of R36 organisms or by passage over columns of Sepha-

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rose phosphorylcholine beads (Table VIII). The level of T15 in a pool of BALB/c normal serum showing a log 2 HI titer 6 was reduced to 0 following absorption by passage over a column containing Sepharose phosphorylcholine beads. Similarly, absorption of BALB/c normal and immune serum with R36

TABLE VII

HI of T15 Idiotype with Normal and AntiPneumococcus (R36A) Sera

Strain	Type of serum*	Total no. sera	No. sera with Log 2 HI titer of:												Mean
			0	1	2	3	4	5	6	7	8	9	10	11	12	
C ♀	Pre	10	—	—	—	—	—	1	—	1	1	3	2	1	—	7.8
C ♀	Post	10	—	—	—	—	—	—	—	—	1	1	2	3	3	10.6
C ♂	Pre	10	—	—	—	—	—	2	3	1	—	1	3	—	—	7.4
C ♂	Post	10	—	—	—	—	—	—	1	—	—	1	3	4	1	10.1
BKa ♀	Pre	10	—	—	3	6	1	—	—	—	—	—	—	—	—	2.8
BKa ♀	Post	10	—	1	2	7	—	—	—	—	—	—	—	—	—	2.6
BKa ♂	Pre	10	—	1	3	5	1	—	—	—	—	—	—	—	—	2.6
	Post	10	—	2	2	6	—	—	—	—	—	—	—	—	—	2.4
CB20 ♀	Pre	22	—	4	13	5	—	—	—	—	—	—	—	—	—	2.0
	Post	22	—	—	1	11	10	—	—	—	—	—	—	—	—	3.4
CB20 ♂	Pre	16	—	2	8	5	1	—	—	—	—	—	—	—	—	2.3
	Post	16	—	—	—	6	9	1	—	—	—	—	—	—	—	3.7
C × BH	Pre	11	9	2	—	—	—	—	—	—	—	—	—	—	—	0.2
	Post	11	—	5	5	1	—	—	—	—	—	—	—	—	—	1.7
C × BI	Pre	9	7	2	—	—	—	—	—	—	—	—	—	—	—	0.2
	Post	9	9	—	—	—	—	—	—	—	—	—	—	—	—	0.0
AL	Pre	10	9	1	—	—	—	—	—	—	—	—	—	—	—	0.1
	Post	10	10	—	—	—	—	—	—	—	—	—	—	—	—	0.0

* All sera were absorbed with SRBC before the HI test. Pre = normal serum from mice before immunization with R36A; Post = serum taken on day 7 following i.p. injection of 0.5×10^9 killed R36A organisms.

TABLE VIII

Effect of Absorption of Normal and Immune Serum by R36 Pneumococci Or By Passage Over a Sepharose Phosphorylcholine Column on the T15 Log 2 HI Titer

Strain	Type of serum*	No. of mice	Absorption method	Log 2 HI titer range	
				Preabsorption	Postabsorption
BALB/c	Normal‡	4	R36A Pn§	9–11	0
"	Immune ‡	4	R36A Pn	11–13	0
"	Normal‡	8‖	Seph-Pc-col	6	0
CB20	Normal‡	3	R36A Pn	3	0
"	Immune‡	3	R36A Pn	3–4	0

* The sera were selected for high titers for use in this study.

‡ All inhibitors were preabsorbed with SRBC.

§ R36A is a rough strain of pneumococci.

‖ Pool of serum from eight mice adsorbed on a Sepharose phosphorylcholine column.

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organisms reduced the log 2 mean HI titer of 10 for normal serum to 0, and for immune serum from a mean HI titer of 12 to 0. The log 2 HI titer of T15 found in CB20 strains was also reduced from 3 to 0. These results indicate that the removal of antiphosphorylcholine-binding proteins from serum also removes the molecules containing the T15 idiotype.

DISCUSSION

In a previous study we described a group of phosphorylcholine-binding IgA myeloma proteins that shared the same idiotypic specificity (9). These proteins were derived from five independently induced plasmacytomas in BALB/c mice.

The shared idiotypic specificity (the T15 idiotype) was assigned to the Fab part of the myeloma proteins. In subsequent structural studies it has been found that two of these proteins, T15 and H8, have the same V_H and V_L amino acid sequence through the first hypervariable region of the light and heavy chains (32, footnote 2). Further, no structural differences have been demonstrated as yet between these proteins by peptide map techniques (32, footnote 2) suggesting that the proteins with the T15 idiotype will have a close if not absolute primary structure identity. At this time eight BALB/c plasmacytomas of independent origin have been shown to produce phosphorylcholine-binding IgA myeloma protein with the same (T15) idiotype. Cosenza and Köhler (20, 21) demonstrated that IgM antibody-forming cells in the BALB/c mouse that appeared following an immune response to R36A pneumococci (a phosphorylcholine-containing antigen) also possessed the T15 idiotype, thus relating a normal phosphorylcholine-binding immunoglobulin to a myeloma protein.

In the present study, we have extended these observations by demonstrating that the serum of normal, conventionally raised BALB/c mice contains 8–64 μg/ml of an immunoglobulin that binds phosphorylcholine and possesses the T15 idiotype. The molecules carrying the T15 idiotype were identified by antisera prepared in A/He mice immunized with H8 or T15 myeloma proteins. The A/He anti-T15, H8 antisera curiously lack demonstrable anti-allotypic antibodies and do not resemble the usual antisera prepared in these mice with other BALB/c IgA myeloma proteins that show both idiotypic and allotypic specificities. A/He allotypic antisera prepared to other BALB/c IgA myeloma proteins, however, recognize the allotypic determinants $A^{12, 14}$ on T15 and H8 myeloma proteins (9). This immunoglobin (T15-Ig) is not demonstrable in germ-free BALB/c serum but does appear when germ-free BALB/c mice are conventionalized. Further, immunization of BALB/c mice with R36A pneumococci raises the level of immunoglobulin with the T15 idiotype from approximately log 2 titer of 7 to 10 (Table VII). The presence of high levels of phosphorylcholine-binding T15-Ig in normal serum of virtually every BALB/c mouse we have examined suggests that this is a natural antibody which may

[2] Barstad, P., S. Rudikoff, M. Potter, M. Cohn, and L. Hood. 1973. Manuscript submitted for publication.

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develop in response to the products of microorganisms in the gastrointestinal or respiratory tracts. A variety of microorganisms found in the intestinal tract of mice are known to produce phosphorylcholine-containing antigens (33).

A survey of other inbred mice revealed many strains had no or very little T15-Ig in their serum. Further, immunization of several of these strains (AL/N, C57BL/Ka, CB20, C × BH, and C × BI) with R36A pneumococci produced no or very low titers of T15-Ig. These results suggested genetic differences among the inbred strains that governed the expression of T15-Ig. The low levels of immunoglobulin with the T15 idiotype in C57BL strains permitted a genetic study of the inheritance of the high levels of T15-Ig. This was facilitated by the availability of the Bailey RI strains (seven new inbred strains of mice derived from seven different pairs of [C57BL × BALB/c]F_2 hybrid mice) and a congenic strain of mice CB20 which was developed by introgressively backcrossing the C57BL/Ka IgC_H locus onto the BALB/c background for 20 consecutive generations and then breeding a homozygous stock by brother-sister mating of the 20th backcross progeny. It was demonstrated that high levels of T15-Ig (hi-T15-Ig) were found in Bailey RI strains with the BALB/c IgC_H allotype locus, and low levels of T15-Ig (lo-T15-Ig) were found in Bailey RI strains with C57BL allotype (IgC_H locus) and CB20. Further (BALB/c × C57BL)F_2 mice homozygous for the C57BL IgC_H allotype locus had a lo-T15-Ig while mice homozygous and heterozygous for the BALB/c IgC_H allotype had high levels of T15 Ig (hi-T15 Ig). These results established conclusively that the hi-T15-Ig phenotype was inherited as a Mendelian dominant and further was linked to the BALB/c IgC_H allotype locus.

The strain distribution of the hi-T15-Ig characteristic indicated that only strains carrying the allotype locus $G^{1,6,7,8,}H^{9,11}F^{19}A^{12,13,14}$ (a^1 allotype) possessed the hi-T15-Ig character. Further, not all strains with the a^1 allotype had hi-T15-Ig. The strain distribution of hi-T15-Ig resembles thus far that described for the inheritance of a λ-type $\alpha 1 \rightarrow 3$ dextran immune response reported by Blomberg et al. (Table IX) (15, 16). $\alpha 1 \rightarrow 3$ dextran λ-type antibodies in BALB/c are associated also with an idiotypic determinant found on the J558 IgA protein that binds $\alpha 1 \rightarrow 3$ dextran. Thus far the same inbred and Bailey RI strains that have hi-T15-Ig also have the λ-type-J558-Ig. An exception is the BAB-14. This strain was developed by Herzenberg from our CB backcross stock. These mice are homozygous for C57BL/Ka IgC_H, but are positive for the λ-type-J558-Ig and have the lo-T15-Ig. This raises the possibility that a crossover has occurred in the BAB-14 in which the gene controlling λ-type-J558-Ig and the hi-T15-Ig of BALB/c origin have been dissociated so that the λ-type-J558-Ig gene has become linked to C57BL/Ka IgC_H locus. Weigert (personal communication) has tested our CB20 stock and found that it does not have the λ-type-J558-Ig response. Sher and Cohn (18) have described the genetics of an immune response to phosphorylcholine in various inbred strains of mice. Their study clearly shows two phenotypes that are distributed in

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Summary of IgV-Region Genetic Markers in the Mouse

References	Characteristics of IgV-region marker				Type of idiotypic antisera*	Strains of mice classified according to IgC_H allotypes that produce Ig with the respective V-region marker									
	Antigen-binding specificity	Idiotype on:	L or H chain association			a^1		a^2				a^4		a^5	
			L	H		Hi	Lo	Hi	Lo	Hi	Lo	Hi	Lo	Hi	Lo
15, 16 37, 38	$\alpha 1 \rightarrow 3$ dextran	J558 MP; antibodies to β1355S dextran	λ_1		Allo (A/He)	BALB/c 129 C58 C × BG C × BJ	CBA	BAB14	C57BL/6 SJL/J C × BD C × BE C × BH C × BI C × BK				AKR A/He NZB		
This paper	Phosphorylcholine	T15-S63 group of MP‡: Natural antibody	κ-T15		Allo (A/He)	BALB/c 129 C58 C57L ST C × BG C × BJ	CBA C3H		C57BL/6 C57BL/10 SJL/J B10.D2 BAB14 CB20 C × BD C × BE C × BH C × BI C × BK		DBA/2 RIII		A/He AL/N AKR NZB		NH
18	Phosphorylcholine	S107MP‡ antibodies to pneumococcus polysaccharide			Allo (A/He)	BALB/c CBA		C57BL/10 SJL/J		DBA/2 DBA/1		NZB CAL-9	A/J A/WySn AKR/J		
14, 41	*P*-azophenyl-arsonate(ars)	A/He, anti-ars			Xeno (rabbit)		BALB/c CBA C3H C57BR		C57BL/6 LP SJL/J SM B10·A		DBA/2 RF SWR	A/J¶ A/He¶ AL/N¶ CAL-9 CAL-20	NZB¶ AKR¶		CE
5, 17, 39	Group A streptoccal carbohydrate	A/J anti-A-CHO (clone A5A)			Xeno (guinea pig)		BALB/c C57L**		C57BL/6 SJL/J		SWR DBA/2** DBA/1	A/J			CE
40	NIP§ (4-hydroxy-5-iodo-3 nitrophenacetyl)	None yet demonstrated					CBA BALB/c C3H MA/J C57/L ST/6J IAH	C57BL/6 CB20 C57BL/ks LP C57BL/10							

MP, myeloma protein.

Strains of mice that share a series of common IgC_H determinants are for convenience placed in an allotype group a^1, a^2 etc. See footnote in Table IV for details. Some of the groups, e.g. a^3, a^4, have been further subdivided by minor markers (30).

* Alloantiserum prepared by allogeneic immunization. Xeno, antiserum prepared by xenogeneic immunization.

‡ The myeloma proteins T15, H8, M299, S63, and S107 share common idiotypic determinants.

§ The anti-NIP system is tentatively considered a V-region marker based on unpublished data of O. Mäkela and Imanishi (personal communication). Anti-NIP antibodies are raised by immunization with NIP (4-hydroxy-5 iodo-3 nitro phenacetyl). The anti-NIP antibodies in Hi strains have a higher affinity for NIP than Lo strains.

¶ In the subdivision of the a^4 IgC_H allotype group NZB and A are in one subgroup AL and AKR are in the other (30).

** These strains showed weak cross-reacting specificities with the A5A idiotype.

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strains of mice that are different than those observed in the present study with T15-Ig marker.

The major question presented by these findings is to define the genetic basis for the hi-T15-Ig and lo-T15-Ig phenotypes. The T15-Ig character is clearly a variable-region related function. T15-Ig is antigenically located on the Fab fragment, is related to antigen binding, and is independent of class functions. There is growing evidence in several different systems that the Ig_V genes in the mouse are closely linked to the C_H genes. This was first demonstrated by Pawlak et al (14), who showed that a gene controlling an idiotype found on anti-arsonate antibody raised in strain A/He and AL/N mice was linked to the IgC_H genes in these strains (Table IX). Evidence by Blomberg et al. (15, 16) have further supported this finding with studies on genes controlling a λ-type $\alpha 1 \rightarrow 3$ dextran response (Table IX). Much of this evidence of linkage has depended upon the use of Ig-congenic strains of mice. It is important, therefore to comment briefly on the method for developing congenic strains of mice and on the possible structure of the Ig heavy-chain locus. First, congenic strains are developed by introgressively backcrossing mice, selecting for a specific allotype marker. Since the IgC_H allotype locus in the mouse has not yet been linked to any other known gene it is not known how much "foreign" DNA is being introduced. This could be a very large or a very small segment. Preliminary evidence from hybridization studies utilizing a pure heavy-chain messenger RNA suggests that the V_H locus in the mouse may contain as many as 5,000 genes (34). Thus, the evidence of linkage of IgV-related functions obtained from congenic strains of mice probably indicates a large segment of DNA is introduced during the development of an Ig congenic strain. The same is clearly true in the case of the *H-2* congenic strains. This raises the possibility that the newly introduced chromatin contains more genes than expected and also suggests several possibilities for explaining the genetic basis for the difference between the high (hi) and low (lo) strains.

Possible interpretations are: (*a*) The hi-lo T15-Ig difference is a function of a V_H or V_L or both structural gene differences. (*b*) The hi-lo T15-Ig difference is a function of another gene that interacts with the structural genes and regulates their expression.

Structural Gene Differences.—The hi-lo T15-Ig phenotypic differences are probably controlled by V_H structural gene differences. The evidence for this at present is indirect but nonetheless convincing.

First immunochemical studies have revealed a close association or linkage of corresponding V and C genes. Immunoglobulin L and H polypeptide chains are each controlled by C and V structural genes, i.e., C_H, V_H, C_L, and V_L. It is generally thought that C and V genes are joined to form the template for an H or L polypeptide chain (35, 36). This probably requires a close physical association. Further, C genes are associated with specific sets of V genes and thus far no evidence of exchange of V genes between different C genes, e.g.

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C_κ, C_λ, and the group of C_H genes, has been found. In rabbits heterozygous for C_H and V_H genes, 99% of immunoglobulin synthesis involves genes in the cis arrangement (see 30 for references). Allelic exclusion involves corresponding C and V genes (see 30 for references). These findings suggest then that a C-gene locus has a close association with its corresponding V genes, and that the two genes may be in close genetic linkage. In the mouse, where genetic markers on IgC_L genes have not yet been found, it has not been possible to associate H and L genes and thus it may be argued that an L-chain gene might be responsible for the T15 idiotype. Indeed, a special subclass of V_κ in the mouse has so far only been found in association with the T15-S63 group of phosphorylcholine-binding myeloma proteins (32, footnote 2). A related phenomenon in the λ-type $\alpha 1 \rightarrow 3$ dextran-binding Ig system however, provides the most compelling evidence for assigning the T15 idiotype to V_H instead of V_L. In the $\alpha 1 \rightarrow 3$ dextran system the $\alpha 1 \rightarrow 3$ dextran-binding myeloma proteins J558 and M104E have an L-chain subunit with the identical amino acid sequence (37, 38), but nonetheless have different idiotypes. From this it is assumed that the V_H structure is responsible for the idiotypic determinant but depends however on the availability of a specific V_L subunit. In the $\alpha 1 \rightarrow 3$ dextran system this is the λ-chain; in the phosphorylcholine-T15 system this is the V_κ chain.

Allelic differences between BALB/c and C57BL in the V_H gene subunit of the T15 molecule is probably the most plausible explanation. Here it is postulated that both BALB/c and C57BL have the same homologous V_H gene but that mutations have created differences in the part of the gene that controls its ultimate antigenic idiotypic structure. The T15-like molecules in C57BL are only partially identical to those in BALB/c and complete inhibition of the anti-T15-T15 idiotype system can never be achieved with T15-like molecules of C57BL origin. In the studies presented we never were able to obtain high HI titers with any immunoglobulins derived from C57BL. Thus, an allelic difference in a V_H gene is a very plausible explanation.

A second structural gene difference might be due to the presence of a competing gene. For example, both BALB/c and C57BL have a large number of V_H genes, many of which in each strain can produce V_H polypeptide subunits of phosphorylcholine-binding proteins. BALB/c favors the T15 type which among the many available has the strongest affinity and hence will emerge in any immunization including natural immunization. By contrast, C57BL has another type of antiphosphorylcholine immunoglobulin that utilizes a different V_H gene. The high affinity C57BL antiphosphorylcholine-Ig lacks the T15 idiotype.

Another highly speculative explanation may be related to the process of forming C_H-V_H complexes. It is generally thought there are a few C_H genes each of which can link separately to the many V_H genes to form a DNA template for the Ig heavy chain. The organization of V_H genes on the chromosome may favor the formation of specific C_H-V_H complexes. Hence it is possible that

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the organization of V_H genes in the BALB/c and C57BL are different and could constitute the basis for a difference in the retrieval of the T15 V_H gene. This might be easily achieved in BALB/c and more difficult to attain in C57BL.

Finally, the V_H gene complex may consist of a relatively limited number of gene types each of which are repeated many times. Two complex Ig loci may differ from each other by the number of copies of individual genes. If the T15 V_H gene is highly redundant in BALB/c, high levels of T15-Ig in BALB/c could be a function of the frequent pairing of V_H (T15) with a C_H gene in BALB/c.

Arguments Favoring Other Gene Differences.—With existing data the presence of a regulator gene that controls the levels of a specific type of immunoglobulin such as the T15 Ig cannot be ruled out.

Summary of IgV-Region Markers in the Mouse.—In Table IX we have summarized six different systems in which an IgV-region marker has been involved. Clearly the IgV-region markers, J558 associated λ-type $\alpha 1 \rightarrow 3$ dextran, A/He azo-phenylarsonate-associated idiotype, and the T-15 idiotype have been linked to the IgC_H region by virtue of finding phenotypic differences in Ig-congenic strains of mice. In addition, Eichmann's A/J anti A-CHO idiotypic system also appears to be linked to the IgC_H system based on strain distribution and breeding experiments (5, 17, 39).

The anti-NIP antibodies of high affinity produced by C57BL/6, C57BL/Ks, LP, and C57BL/10 in response to immunization with NIP is another possible system. Thus far the higher affinity anti-NIP antibodies are found only in strains in the a^2 group (41). Recently, Imanishi and Makela (unpublished observations) have found that antibodies in immunized CB20 mice have a high affinity for NIP.

Finally, the phosphorylcholine antibodies carrying the S107 idiotype induced by immunization with pneumococcus C polysaccharide (PnC), or SRBC-PnC complexes have a very different strain distribution than the T15-Ig idiotype. At present we have no explanation for the difference between this system and ours. Both appear to involve phosphorylcholine-binding antibodies with idiotypes related to the T15-S63 group of myeloma proteins. It is possible that the difference is related to the mode of immunization; in our system we are dealing with natural antibodies while in the Sher and Cohn (18) system the antibodies were induced by immunization with PnC.

SUMMARY

The idiotype present on the Fab of a phosphorylcholine-binding IgA myeloma protein TEPC 15 (T15) of BALB/c origin was found in normal serum of BALB/c mice. Molecules carrying the T15 idiotype in normal serum could be adsorbed with Sepharose phosphorylcholine beads and R36A pneumococci. The T15 idiotype is absent in germ-free BALB/c but appears when the mice are conventionalized. A survey of normal sera of inbred strains for the T15 idiotype showed it to be present in BALB/c, 129, C57L, C58, and ST and absent or in low levels in CBA, C3H, C57BL/6, C57BL/Ka, C57BL/10, SJL, B10.D2,

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DBA/2, RIII, A, AL, AKR, NZB, and NH inbred strains of mice. The T15 idiotype is associated with some but not all strains carrying the IgC_H allotypes found in BALB/c. Linkage of genes controlling the T15 idiotype in normal serum to the IgC_H locus of BALB/c was demonstrated in F_2 progeny of a BALB/c and C57BL cross, Bailey's recombinant inbred strains, C × BD, C × BE, C × BG, C × BH, C × BI, C × BJ, C × BK, and CB20 congenic strains. Among these strains, only those possessing the IgC_H locus of BALB/c including the F_2 progeny consisting of BALB/c homozygotes and BALB/c/C57BL heterozygotes and C × BG and C × BJ recombinants showed the T15 idiotype.

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