CLONAL NATURE OF THE IMMUNE RESPONSE TO PHOSPHORYLCHOLINE

IV. Idiotypic Uniformity of Binding Site-Associated Antigenic Determinants Among Mouse Antiphosphorylcholine Antibodies*

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Studies of the binding characteristics for a number of choline analogues have revealed that antiphosphorylcholine (PC)[1] antibodies from 15 different inbred mouse strains which differ in histocompatibility and heavy-chain allotype are remarkably restricted and show the same binding specificity as a PC-binding myeloma protein, HOPC 8.[2, 3] This suggests that the anti-PC antibodies of all mice possess similar binding sites for PC. Indeed Cosenza and Köhler (5) and others (2, 6, 7) using mouse antimouse idiotypic antisera have clearly demonstrated that anti-PC antibody from BALB/c possesses antigenic determinants shared by HOPC 8. Paradoxically however, antibodies from other strains, such as C57BL/6, A, and CE mice (2, 5–7), do not have this idiotypic specificity even though their antibody specificity cannot be distinguished from that of BALB/c. These studies indicate that variable-region determinants differ in the anti-PC antibodies of different strains of mice, but since the hapten inhibition of binding to idiotype was not studied, it is not clear that these idiotypic differences lie in the binding region. This consideration is crucial to the analysis of antibody combining-site diversity since selective pressures exerted on binding site and nonbinding site regions may be different.

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[1] *Abbreviations used in this paper:* BBS, borate-buffered saline; BSA, bovine serum albumin; C, choline; GPC, L-α-glycerophosphorycholine; HI, hemagglutination-inhibition; IgM_s, IgM subunits; NMG, normal mouse globulin; PBS, phosphate-buffered saline; PC, phosphorycholine; TBA, tube-binding assay.

[2] This protein is one of a number which have the same binding specificity (1, 2), identical *N*-terminal V_H and V_L sequences (3) and, as demonstrated by mouse antimouse antisera, serologically identical idiotypes (4).

[3] Claflin, J. L., and J. M. Davie. 1974. Clonal nature of the immune response to phosphorylcholine. III. Species-specific binding characteristics of rodent antiphosphorylcholine antibodies. Manuscript. Submitted for publication.

We have approached this problem by preparing heterologous, site-specific antisera to HOPC 8 to probe for binding site uniformity among anti-PC antibodies raised in different mouse strains. The idiotypic antibody, prepared in rabbits and isolated from an HOPC 8-immunoadsorbent by elution with PC, fails to distinguish among anti-PC antibodies from all strains of mice tested, regardless of genetic background. Thus, the idiotypic determinant(s) described here appears to be directly related to those binding-site regions on anti-PC antibodies with similar specificities for choline analogues, and these regions are regularly expressed in all mouse strains. Antibodies of other specificities raised in mice or anti-PC antibodies raised in other rodent species lack this antigenic determinant.

Materials and Methods

Reagents.—Phosphorylcholine (PC), L-α-glycerophosphorylcholine (GPC), and choline (C) were obtained from Sigma Chemical Co., St. Louis, Mo. The calcium and cadmium ions in PC and GPC, respectively, were precipitated with phosphate before use.

Animals.—BALB/c, CBA, C57L, C58, C57BL/6, SJL, DBA/1, I/Ln, SWR, A, AKR, and CE mice were obtained from Jackson Laboratories, Bar Harbor Maine; P/JN, RIII/AnN, and NH/LWN strains from Dr. Carl Hansen, Genetics Research Unit, NIH, Bethesda, Md.; C3H/Sn mice from a colony maintained by Dr. R. Graff at the Jewish Hospital of St. Louis; and strain 129 from Dr. V. Suntzeff, Washington University School of Medicine. Wild *Mus musculus* were trapped on two separate local farms. Golden Syrian hamsters and inbred Wistar/Furth rats were purchased from ARS/Sprague-Dawley, Madison, Wis. Outbred guinea pigs and rabbits were obtained from Eldridge Rabbitry, St. Louis, Mo. The deer mice, *Peromyscus maniculatus artemisiae*, a gift from Dr. John Coe, were derived from a random bred colony maintained at the Rocky Mountian Laboratory, NIAID, Hamilton, Mont.

Plasmacytomas.—The plasma cell tumors, HOPC 8, TEPC 15, McPC 603, MOPC 167, MOPC 511, MOPC 460, MOPC 315, MOPC 104, MOPC 70, LPC 1, and MOPC 195 were obtained from Dr. M. Potter, National Cancer Institute, NIH Bethesda, Md. These tumors were maintained by serial passage of tumor cells in BALB/c mice.

Immunologic Reagents.—PC-coupled sheep erythrocytes (SRBC) were prepared as previously described (2) using *p*-diazonium phenylphosphorylcholine. Antisera specific for mouse κ, λ, and μ chains were prepared in goats and rendered specific by adsorption to Sepharose-protein (8) immunoabsorbents.

Immunization and Measurement of Anti-PC Antibody.—Antibodies to PC were raised by single or multiple intravenous injections of 10^8 heat-killed (56°C, 30 min) *Diplococcus pneumoniae* strain R36A (9). This organism contains PC as a cell wall component (10). PC-specific serum antibodies and plaque-forming cells were detected as previously described by the hemagglutination reaction and Jerne plaque assay using PC-SRBC as the indicator erythrocytes (2). Antisera to dinitrophenyl-keyhole limpet hemocyanin (DNP-KLH) with high titer IgM antibody were obtained from BALB/c and A mice 4 days after the second of two injections (spaced by 7 days) of 100 μg antigen in incomplete Freund's adjuvant. AKR antisera specific for Group A streptococcal carbohydrate was a gift from Dr. D. Briles of this laboratory.

Purification of Myeloma Proteins and Specific Antibodies.—Immunoglobulins with specificity for PC were isolated by affinity chromatography as described by Chesebro and Metzger (11). Briefly, serum from immunized mice or ascites fluid containing mildly reduced and alkylated myeloma proteins were passed over a PC-Sepharose immunoadsorbent. After washing with 0.20 M borate, 0.15 M NaCl buffer, pH 8.0 (BBS) to remove unbound protein, the specific protein was eluted with 10^{-3} M PC. The eluate was dialyzed extensively and recycled over the

PC-immunoadsorbent. The isolated mouse anti-PC antibodies were IgM by immunoelectrophoresis and by immunodiffusion with class-specific antisera. In some instances, purified IgM was reduced with 0.02 M cysteine to give IgM subunits (IgMs) (12). The conversion to IgMs was monitored by disc electrophoresis in polyacrylamide gels (13) and shown to be >95% complete in 30 min. Normal mouse globulin (NMG) was prepared from pooled normal BALB/c serum by DEAE-cellulose chromatography (14); as shown by immunoelectrophoresis, this fraction contained IgM, IgA, IgG, and minor contamination by alpha globulins.

Idiotypic Antisera.—The preparation and characterization of the two idiotypic antisera specific for HOPC 8 will be described in detail elsewhere.[4] One antiserum, called A/J anti-H8, was produced in A/J mice by conventional immunization with HOPC 8 (15). Allotypic antibody was removed by adsorption to a MOPC 460-Sepharose immunoadsorbent (8, 9). The second antiserum was prepared by heterologous immunization of rabbits with HOPC 8 protein and subsequent isolation of those antibodies with specificity for the binding-site region of HOPC 8.[4] Briefly, antiserum was collected from a rabbit (R2) 10 days after the second of two injections of HOPC 8 protein emulsified in complete Freund's adjuvant, diluted in an equal vol of BBS and passed over an HOPC 8-Sepharose immunoadsorbent column (8). After washing the column with excess BSS to remove unbound protein, 10^{-3} M PC in BBS was added and the effluent was collected, concentrated, and dialyzed against BBS. This preparation (R2 anti-$H8_s$) is idiotypically specific for HOPC 8 and contains antibody directed to HOPC 8 binding-site determinants.[4]

Detection of Idiotypic Determinants.—Two different assays, hemagglutination-inhibition (HI) and solid-phase radioimmunoassay, were usually run in parallel to detect idiotypic specificities.

Hemagglutination-Inhibition: Idiotypic antiserum was titered against HOPC 8-coated SRBC and a dilution of antiserum fourfold less than the hemagglutination endpoint was determined. Normal sera, immune sera, or haptens were tested for their ability to inhibit the hemagglutination of H8-SRBC by the dilution of anti-idiotypic antisera. Inhibition by sera suggests the presence of immunoglobulins sharing antigenic specificities with HOPC 8.

Solid-Phase Radioimmunoassay: The tube-binding assay (TBA), described by Askenase and Leonard (16) and modified by us[4], was used as a means of quantitating idiotypic cross-reactions. Micro-test tubes (Beckman Instruments, Inc., Fullerton, Calif.) were coated with 0.2 ml of anti-idiotypic serum diluted in 0.15 M NaCl, 0.005 M phosphate buffer, pH 7.4 PBS. After 4 h at room temperature, the antiserum was aspirated, the tubes washed two times with PBS and filled with 1% bovine serum albumin (BSA) in PBS. At the time of assay (within 4 days after addition of BSA) the BSA solution was removed and 0.21 ml of [^{125}I]HOPC 8 (~6,000 cpm) in PBS containing 1% BSA, 0.1% normal mouse serum, and 0.2% sodium azide was added. Tubes were incubated for 16–18 h at 37°C at which time the contents were aspirated, the tubes rinsed with PBS and the radioactivity bound measured in a gamma counter. Four different preparations of [^{125}I]HOPC 8 (17) were used in these experiments and ranged in specific activity from 16–34 μCi/μg. Specific binding to antibody-coated tubes and nonspecific binding to control tubes was 25–35% and 0.3–0.5%, respectively, of added radioactivity.

RESULTS

Specificity of Idiotypic Antisera for HOPC 8.—The isolation and characteristics of A/J anti-H8 and R2 anti-$H8_s$ sera are described in detail elsewhere.[4] Experimental results demonstrating the specificity of these antisera are summarized in Fig. 1 and Table I. By both the HI and the TBA the antisera were

[4] Claflin, J. L., and J. M. Davie. 1974. Specific isolation and characterization of antibody directed to binding site antigenic determinants. Manuscript submitted for publication.

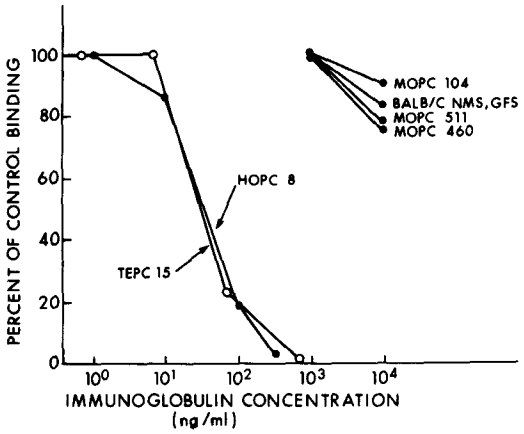

FIG. 1. Specificity of idiotypic antisera. Inhibition of binding of [^{125}I]HOPC 8 to antibody-coated tubes by NMG and germ-free serum (GFS) from BALB/c mice and the myeloma proteins HOPC 8, TEPC 15, MOPC 511 and MOPC 460 (all α, κ) and by MOPC 104 (μ, λ) is tested.

TABLE I

Specificity of Anti-HOPC 8 Idiotypic Antisera

Inhibitor	HI titer	
	R2 anti-H8$_8$	A/J anti-H8
	mg/ml	*mg/ml*
BALB/c NMS	>2*	>2
HOPC 8	0.000081	0.000081
TEPC 15	0.000040	0.000020
MOPC 460, MOPC 315, MOPC 104, MOPC 70, LPC 1, MOPC 195	>2	>2
MOPC 511, MOPC 167, McPC 603	>2	>2
PC	$10^{-3.5}/10^{-4.5}$‡	$10^{-2}/10^{-3}$
GPC	$10^{-2.5}/10^{-3.5}$	$>10^{-2}/10^{-2}$
C	$>10^{-1.5}/10^{-2}$	—§

* Mean determined from three separate experiments. Data represents concentration of protein giving complete inhibition.

‡ Molar concentration giving complete HI/molar concentration giving no HI.

§ No HI detected at $10^{-1.5}$ M choline.

idiotypically specific for HOPC 8 and TEPC 15.[1] Approximately 10–30 ng/ml and 20–81 ng/ml of HOPC 8 or TEPC 15 gave complete inhibition in the TBA and HI tests respectively. Normal serum or myeloma proteins, including PC-binding myelomas with antibody specificity different from HOPC 8 (1, 2), at > 1,000 times higher concentrations, gave only marginal inhibition.

Inhibition of binding was also accomplished with choline analogues (Fig. 2

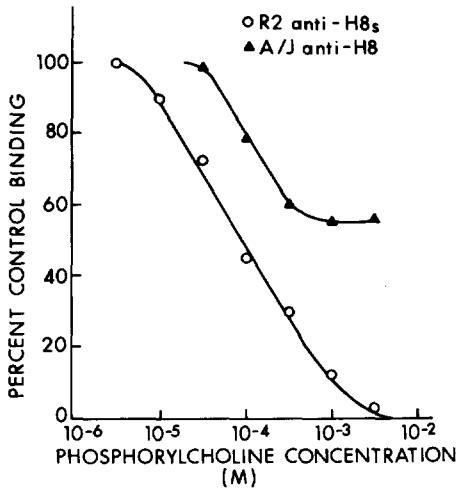

FIG. 2. Hapten inhibition of binding to idiotype. Inhibition of binding of [^{125}I]HOPC 8 to R2 anti-H8$_s$ (○)- or A/J anti-H8 (△)-coated tubes by phosphorylcholine was measured in a TBA.

and Table I). In both assays, the reaction between R2-anti-H8$_s$ was completely inhibited by PC and the inhibition pattern obtained with PC, GPC, and C reflected the specificity of HOPC 8 for these haptens (1, 2). The binding of A/J anti-H8 was only partially inhibited by the choline haptens and as indicated in the TBA about 50% of the antibody was specific for determinants of HOPC 8 not associated with the binding site. Thus, in both assays each antiserum was idiotypically specific but only the heterologous antiserum was exclusively site directed.

Idiotypic Specificities on Mouse Anti-PC Antibody.—Using mouse (A or CE) antisera, studies in several laboratories (2, 5–7) have demonstrated that anti-PC antibodies raised in BALB/c but not A or CE mice share idiotypic specificities with HOPC 8. We have shown that the rabbit anti-HOPC 8 antiserum, R2-anti-H8$_s$, recognized antigenic determinant(s) on BALB/c anti-PC antibodies.[4] These experiments are expanded here to include other strains of mice, selected for differences in allotype and *H-2* type. The results presented in Table II show that the two idiotypic antisera recognize different determinants on anti-PC antibodies of mice. The mouse anti-H8, as shown elsewhere (2,7), distinguishes anti-PC antibody raised in BALB/c and C58 from the anti-PC antibody produced in C57BL/6, DBA/2, A, and CE. By contrast the rabbit anti-H8$_s$ recognizes determinant(s) on the anti-PC antibodies produced in each of the strains of mice. Sera from unimmunized mice and control sera containing high titer IgM and IgG antibodies of different specificities do not inhibit the HA.

Association of Idiotype with Anti-PC Antibody Titer.—The association of H8$_s$ idiotypic determinant(s) with anti-PC antibody was studied in BALB/c

and A mice responding to PC. At different times after immunization individual mice were bled and the titers of anti-PC antibody and of immunoglobulin (Ig)-bearing HOPC 8 idiotypic specificities(s) were determined (Fig. 3). Before injection of pneumococci neither BALB/c nor A mice had detectable levels of

TABLE II

*Idiotypic Specificity of Mouse Anti-PC Antibody**

Serum	Strain	IgC_H‡ Type	HI titer§	
			R2 anti-$H8_s$	A/J anti-H8
NMS	BALB/c, C58, C57BL/6, DBA/2, A, CE	1, 1, 2	<3	<3
		3, 4, 5		
Anti-PC	BALB/c	1	6.1 ± 0.5	7 ± 0
	C58	1	11.0 ± 0.7	8 ± 0.8
	C57BL/6	2	7.7 ± 0.3	<3
	DBA/2	3	6.5 ± 0.3	<3
	A	4	8.0 ± 0	<3
	CE	5	9.7 ± 0.6	<3
Anti-group A	AKR	4	<3	<3
Anti-DNP-KLH	BALB/c	1	<3	<3
Anti-DNP-KLH	A	4	<3	<3

* Sera were obtained 4 days after two biweekly injections of 10^8 pneumococci. See Materials and Methods for preparation of antisera of other specificities. The data represent the mean of the individual responses of three–five animals/group.

‡ Ig heavy-chain linkage groups (15).

§ Reciprocal of the $\log_2$ dilution.

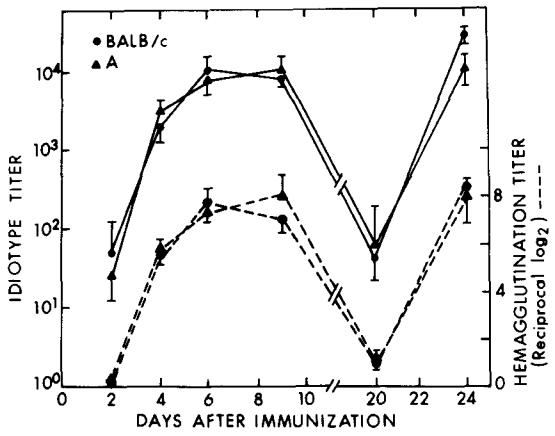

FIG. 3. Association of $H8_s$ idiotype with anti-PC serum titer. Anti-PC hemagglutinin (----) and $H8_s$ idiotype (——) titers were measured in sera from BALB/c and A mice following two injections of 10^8 pneumococci. Idiotype titer is the reciprocal serum dilution giving 50% inhibition of binding of [^{125}I]HOPC 8 to R2 anti-$H8_s$. Data represent mean ± SEM of four mice/group.

anti-PC antibody or appreciable levels of Ig sharing antigenic specificities with HOPC 8. Immunization stimulated a sharp increase followed by a gradual fall in Ig-bearing HOPC 8 determinants, a fluctuation which corresponded directly with the anti-PC titer. Thus in BALB/c and A mice the idiotypic determinant(s) recognized by R2 anti-$H8_s$ only appeared after immunization and correlated with the amount of antibody specific for PC.

The association of idiotypic specificities detected by R2 anti-$H8_s$ with anti-PC antibodies was examined in inbred mouse strains differing in allotype and at the major histocompatibility locus. The mice used in this experiment, though of different genetic backgrounds, produce antibody with the same, restricted binding characteristics for different choline analogues.[3] As indicated in Table III, all strains tested produced high titer anti-PC antibody-bearing idiotypic determinant(s) recognized by R2 anti-$H8_s$. Moreover, the HI titers correlated directly with the anti-PC titers and supported the association of this idiotype with anti-PC antibody. More importantly these results demonstrated that among the strains, even though they differed in IgC_H and *H-2* type, there exists a considerable degree of antigenic similarity in the binding site region among all mouse anti-PC antibodies.

Extent of Shared Idiotypy on HOPC 8 and Mouse Anti-PC Antibodies.—In an attempt to quantitate the extent of cross-idiotypic specificity among mouse antibodies and HOPC 8, purified IgM anti-PC antibodies were isolated by

TABLE III

Strain Distribution of $H8_s$ Idiotype

Strain*	IgC_H‡ type	*H-2* type	Anti-PC titer§	R2 Anti-$H8_s$, HI titer‖
BALB/c	1	d	11.3 ± 0.3	10.3 ± 0.7
C3H	1	k	10 ± 0	9.0 ± 0
C57L	1	b	10 ± 0.6	10.3 ± 0.7
C58	1	k	9.3 ± 0.8	8.5 ± 0.3
129	1	b	9.5 ± 0.5	8.0 ± 0.3
P	1	p	10.3 ± 0.5	10.5 ± 0.5
C57BL/6	2	b	16.0 ± 0	16.0 ± 0
SJL	2	s	11.7 ± 0.3	11.3 ± 0.7
DBA/1	3	q	9.3 ± 0.3	8.3 ± 0.3
RIII	3	r	9.0 ± 0	9.0 ± 0
SWR	3	b	8.0 ± 1	7.7 ± 0.9
A	4	a	15.0 ± 0	14.5 ± 0.6
AKR	4	k	13.5 ± 0.3	13.3 ± 0.3
NH	5	?	11.5 ± 0.3	11.5 ± 0.3
CE	5	k	11.0 ± 0.6	11.0 ± 0.6

* Sera from three–five mice/group were obtained 4 days after the second injection of pneumococci.

‡ Ig heavy-chain linkage groups (15).

§ Reciprocal $\log_2$ hemagglutination titer obtained with PC-SRC. Sera from unimmunized mice had anti-PC and HI titers <3.

‖ Reciprocal $\log_2$ HI titer determined with R2 anti-$H8_s$ idiotypic antiserum.

affinity chromatography from pooled mouse sera and reduced to IgMs. Each purified antibody was tested for its ability to inhibit the binding of [^{125}I]HOPC 8 to R2 anti-H8$_s$. The results depicted in Fig. 4 (left panel), show that not only are the H8$_s$ idiotypic determinant(s) on mouse anti-PC antibodies but that they are remarkably similar to those on HOPC 8. Each isolated antibody preparation, on a weight basis, gave inhibition comparable to that obtained with HOPC 8. If the determinants shared by these antibodies are identical or very similar to those on HOPC 8, the inhibition of binding of [^{125}I]antibody should give results similar to those obtained by inhibition of [^{125}I]HOPC 8. As indicated in Fig. 4 (right panel), binding of [^{125}I]A anti-PC to R2 anti-H8$_s$ was inhibited equally well by purified mouse anti-PC antibodies and HOPC 8 protein and the mean concentrations giving 50% inhibition (17–49 ng/ml) were very similar to

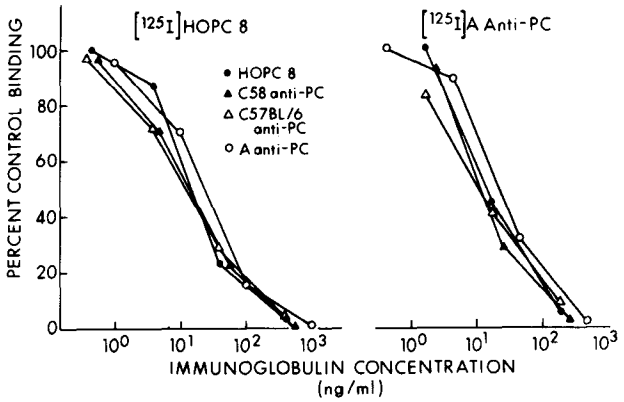

FIG. 4. Idiotypic similarity of H8$_s$ idiotype in HOPC 8 and in mouse anti-PC antibodies.

those (12–28 ng/ml) observed with [^{125}I]HOPC 8 binding. Similar results (not shown) were obtained with ^{125}I-labeled BALB/c and C57BL/6 anti-PC. Though these data do not necessarily indicate idiotypic identity, they do provide strong support for the close similarity of binding site-associated determinants among mouse anti-PC antibodies.

Species Specificity of Idiotypic Antisera.—In another publication, we demonstrated that wild and all inbred strains of *M. musculus* displayed the same restricted binding pattern for various choline analogues and that other rodents possessed species-specific binding patterns.[3] If R2 anti-H8$_s$ is specific for mouse anti-PC binding regions, it should fail to recognize PC-binding regions on antibodies from other rodents. Sera from individual rodents were obtained 4–5 days after two biweekly injections of pneumococci and tested in the TBA for inhibition of [^{125}I]HOPC 8 binding to R2 anti-H8$_s$. The results, shown in Fig. 5, demonstrate that only serum from *M. musculus* contains antibodies that bear idiotypic specificities recognized by R2 anti-H8$_s$. Sera from other rodents,

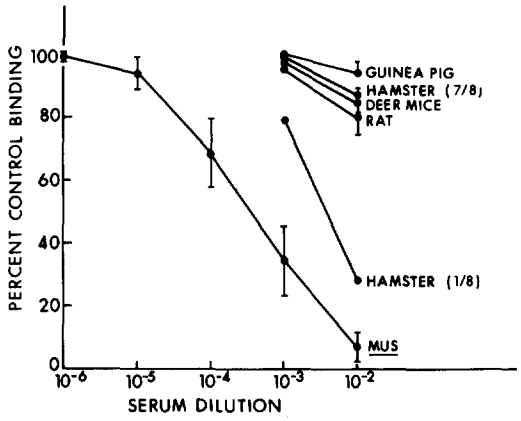

FIG. 5. Species-specificity of $H8_s$ idiotype. Inhibition of binding of [^{125}I]HOPC 8 to R2 anti-$H8_s$-coated tubes by immune serum from wild *M. musculus* (mean HA for PC-SRC of 512) and by immune serum from guinea pigs (mean HA = 640), hamsters (mean HA of 7 = 320 and of 1 = 160), deer mice (mean HA = 640), and Wistar rats (mean HA = 640) was measured. The data represent the mean ± SEM of individual determination of four–eight animals.

even though containing comparable levels of anti-PC antibody, fail to give significant inhibition in the TBA. An exception is the unexplained and repeatable result obtained with one of eight hamsters.

DISCUSSION

This study demonstrates the existence of two probably unrelated antigenic determinants on anti-PC antibodies of inbred mice. One of these determinants is expressed equivalently on anti-PC antibodies of each of 15 strains examined; the other is expressed by only a few strains. Moreover, the species-specific idiotypic determinant appears to represent specificities in the combining site of these antibodies. These results thus directly support findings reported elsewhere[3] which show that all inbred mice produce anti-PC antibody with indistinguishable binding specificities.

The degree of expression of the common idiotypic determinant(s) defined by a rabbit anti-idiotypic antiserum is remarkable and contrasts sharply with that observed in other immune response systems. Thus, each animal in a strain expressed the idiotype, and the extent of expression (titer of idiotype) correlated directly with the amount of anti-PC antibody present. This association was observed regardless of strain or antibody titer and occurred throughout a primary and secondary response. This contrasts with other systems, including that defined with the mouse antisera in the present work, in which intra- and interstrain variabilities in expression of an idiotype are usual (18–23). Köhler has recent evidence in the in vitro primary anti-PC response which supports the

findings presented here. Thus, rabbit antimouse idiotypic antiserum suppresses the anti-PC immune response by spleen cell cultures of both A and BALB/c mice, whereas mouse antimouse idiotypic antiserum suppresses only the response of spleen cells from BALB/c mice (H. Köhler, personal communication).

The evidence provided in this and another paper[4] places the idiotypic determinant detected with the rabbit antiserum in association with the binding site of the antibody. In the first place, the antiserum was absorbed to select only those antibodies reactive with the binding site. Accordingly, the antibody preparation was completely hapten inhibitable and did not react with affinity labeled HOPC 8.[4] More importantly, the antiserum bound only those antibodies exhibiting the HOPC 8-type specificity for the various choline ligands. Ig having specificities different than HOPC 8, which included three unrelated PC-binding myeloma proteins and anti-PC antibody obtained from rats, deer mice, guinea pigs, and hamsters (with one unexplained exception), were not bound. Of additional relevance is a PC-binding myeloma induced in BALB/c·C57BL/Ka (IgC_H)-CB20 mice by M. Potter (National Cancer Institute, NIH, Bethesda, Md.) which carries the IgA allotypic determinant of C57BL/6. This tumor protein has the same binding specificity as HOPC 8 and carries the $H8_s$ determinant. It does not possess, however, the idiotypic determinant recognized by A anti-H8.[5]

The fact that the $H8_s$ idiotype is present on anti-PC antibodies of all mice, and thus can be considered a species-specific idiotype, clearly distinguishes the immune response to PC from other antibody responses. In systems such as the antistreptococcal and anti-*p*-azohapten antibody response idiotypic cross reactions are not usually observed except among selectively bred rabbits (18, 22, 24) or within an inbred strain of mice (20, 21, 23, 25). Nisonoff and colleagues (21), however, have described strong idiotypic cross-reactions among anti-*p*-azobenzoate antibodies from BALB/c, A, and C57BL/6 mice, though the relationship of these idiotypes to combining sites was not discussed. Briles and Krause (26) have recently described two different idiotypes which are present on antistreptococcal antibodies originating in BALB/c (IgC_H group 1), C57BL/6 and SJL (IgC_H group 2), SWR and RF (IgC_H group 3), and A (IgC_H group 4) mice. Of paramount importance is the fact that the anti-idiotypic reactions were hapten inhibitable. Another hapten-inhibitable idiotype reported by them was strain specific.

Shared idiotypic specificities are more commonly observed in antibody responses of limited heterogeneity. Thus, in some, but not all strains of mice, antibodies produced to α-1,3-linked dextrans (27–28) or PC (6, 7) exhibit cross-idiotypic specificity and in the former response, the idiotypic specificity appears to be site related (29). Cross-idiotypic specificity also exists within groups of myeloma proteins with similar specificities (30–32), and in some of

[5] Claflin, J. L., S. Rudikoff, M. Potter, and J. M. Davie. Structural, functional, and idiotypic characteristics of a phosphorylcholine-binding IgA myeloma protein of C57BL/ka allotype. Manuscript submitted for publication.

these systems as well as others, structure-function (33–35) or idiotype-function (29, 36) relationships are currently being delineated. Among the human IgM proteins with anti-γ-globulin activity which have been extensively studied by Kunkel and his colleagues, two major groups appear which are classified on the basis of shared idiotypic determinants (32). Two proteins in the Po group, though having light chains belonging to different V_K subgroups (33), exhibit striking heavy-chain sequence similarities through all four hypervariable regions (37). In the other, the Wa group, striking antigenic (V_{KIII} sub-subgroup B) and sequence similarities are seen in the light chains (38). Of additional interest are the antigalactan-binding mouse myeloma proteins described by Rudikoff and Potter (39). These proteins have non-cross-reacting idiotypic determinants but virtually identical sequences from 1 to 23 on the light chain and from 1 to 30 on the heavy chain. These proteins could provide one of the best models for examining structure-function-idiotype relationships. Thus, shared idiotypy is not an infrequent occurrence and, in some cases, an association with antibody specificity has been demonstrated.

In conclusion, we feel that the uniformity of binding regions expressed by mouse anti-PC antibodies is best interpreted as evidence for a common genetic origin of at least the predominant PC-specific clone(s) in mice. The small amount of variability detected in variable regions of different inbred strains points to the marked conservation of these regions with time. While this is consistent with a germ line or multigene theory of antibody diversity, it is also consistent with a somatic mutation or paucigene theory in which the capacity of individual clones to undergo mutation upon antigenic stimulation varies from clone to clone. It is expected that additional insight into the extent and mechanisms of antibody diversification will be gained from structural analysis of anti-PC antibodies.

SUMMARY

A new idiotypic determinant(s) on mouse anti-PC antibodies is described. Antibodies to the determinant(s) were raised in rabbits by immunization with HOPC 8, a PC-binding myeloma protein, and were isolated from HOPC 8 immunoadsorbent by elution with PC. These antibodies react with binding site determinants on anti-PC antibodies raised in all 15 inbred mouse strains tested regardless of histocompatibility or allotype, but fail to react with antibodies of other specificities or with anti-PC antibodies raised in other rodent species. These results correlate closely with other studies[3] which show similar binding specificity of anti-PC antibodies raised in 17 different strains of mice. The site-associated idiotypic determinant(s) is clearly distinct from that detected by mouse anti-HOPC 8 antisera. This latter determinant(s) is present on anti-PC antibodies of only a few strains of mice and may not be in the binding site.

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