DISTRIBUTION OF A CROSS-REACTIVE IDIOTYPIC SPECIFICITY IN INBRED STRAINS OF MICE*

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Idiotypic determinants are present on myeloma proteins (1) and on induced antibodies (2, 3), where they are characterized by their association with antibody of a given specificity. Shared determinants are ordinarily not detectable in nonspecific IgG,[1] in preimmune serum, nor in antibodies of other specificities from the same animal. As would be predicted, idiotypic determinants are localized in the Fab fragment of immunoglobulin molecules (6). In many antibodies the region of the active site appears to be an important idiotypic determinant, as shown by the capacity of specific haptens to inhibit the reaction with anti-idiotypic antibody (7–10).[2] Complete blocking by haptens is generally not observed, which suggests the presence of additional idiotypic determinants on the Fab fragment. This is supported by the capacity of Fab fragments from some, although not all, immunoglobulins to form specific precipitates with their anti-idiotypic antibodies (11); this property would require antigenic multivalence.

Mice of an inbred strain are frequently observed to produce antibodies of a given specificity with shared idiotype. Intrastrain cross-reactions have been observed among the anti-phosphorylcholine (anti-PC)[3] antibodies of different BALB/c mice (12, 13), the anti-*p*-azobenzoate antibodies of A/J mice (14), A/J anti-*p*-azophenylarsonate (anti-Ar) antibodies (14), BALB/c anti-*p*-azobenzoate antibodies (14), BALB/c anti-α,1,3-dextran antibodies (15), and A/J antistreptococcal carbohydrate antibodies (16). In contrast, intrastrain idiotypic cross-reactions among the anti-*p*-

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[1] Although nonspecific IgG can sometimes inhibit precipitin reactions of anti-idiotypic antibodies directed to a myeloma protein, each myeloma protein appears to have at least one idiotypic determinant that is not detectable, by highly sensitive methods, in nonspecific IgG (4, 5).

[2] The question of a possible conformational change is considered elsewhere (7). If such a change does occur it must be greatly restricted in scope since it does not affect anti-Fab or antiallotypic antibodies directed to determinants in the Fab fragment.

[3] *Abbreviations used in this paper:* anti-Ar, anti-*p*-azophenylarsonate; anti-PC, antiphosphorylcholine; B cell, bone marrow-derived cell; BGG, bovine gamma globulin; BSA, bovine serum albumin; CRI, cross-reactive idiotype; KLH, keyhole limpet hemocyanin; SRBC, sheep erythrocytes; T cell, thymus-derived cell.

azophenylarsonate antibodies of C57BL mice are very weak (14) and other examples of poor intrastrain cross-reactions have been observed.[4]

The strongest intrastrain cross-reactions so far noted in our laboratory are those of anti-Ar antibodies of A/J mice; the antibodies of each of more than 100 individual animals tested have been found to share idiotypic specificity. The idiotype of A/J anti-Ar antibodies is, however, present in far lower concentrations in the anti-Ar antibodies of certain other strains of mice (14, 17, and the present investigation). This suggested that idiotype could represent a genetic marker for the variable regions of mouse immunoglobulin polypeptide chains (14, 17), and the information was used to demonstrate linkage of the anti-Ar idiotype to heavy chain allotype (17–19). In this paper we present quantitative data on cross-reactions of the A/J anti-Ar idiotype in 16 inbred strains and 4 congenic strains of mice. The data are discussed in terms of the correlation of idiotype with histocompatibility type and allotype.

Materials and Methods

Inbred Mice.—With the exception of AL/N and BC9 mice, which were the gift of Dr. Michael Potter, all inbred, F_1, and congenic mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. The BC9 strain was obtained by introgressively backcrossing the $Ig C_H$ complex locus of strain AL/N mice onto a BALB/c background. After nine backcross generations the mice were rendered homozygous for the heavy chain allotype of the AL/N strain. A.Sw/Sn and A.By/Sn are congenic resistant strains with the *H-2* type of Sw or By on a strain A background.The B10.A strain has the *H-2* type of an A mouse on a C57BL/10J background.

Preparation of Antisera.—The preparation of the antigen, keyhole limpet hemocyanin (KLH)-*p*-azophenylarsonate, has been described elsewhere (14). Mice were immunized by injecting intraperitoneally 500 μg of this conjugate, emulsified in complete Freund's adjuvant, twice with an interval of 2 wk between injections. This was followed by two biweekly intraperitoneal injections of the antigen in Freund's incomplete adjuvant. Subsequently, boosters were given as necessary at intervals of at least 1 mo to maintain a high titer of anti-Ar antibodies. An ascites fluid was induced by the method of Sommerville (20) in those mice whose antibodies were to be used for the production of anti-idiotypic antisera. The amount of anti-Ar antibody in each antiserum or ascites fluid was determined by the quantitative precipitin reaction using 20–50 μl portions of antiserum per test. The optical density at 280 nm of the washed precipitate, dissolved in 0.04 M NaOH, was determined in quartz microcells.

Methods used for the preparation and absorption of rabbit anti-idiotypic antisera, and for the specific purification of the mouse anti-Ar antibody to be labeled and used in indirect precipitation tests, have been described (14). Binding of the ^{125}I-labeled, specifically purified anti-Ar antibody to its anti-idiotypic antibodies was quantified by an indirect precipitation (antiglobulin) assay, using 0.01 μg of labeled ligand per test (14). In each series controls were run in which rabbit antiovalbumin antiserum was substituted for anti-idiotypic antiserum. The blanks thus obtained (6–10%) were subtracted from experimental data. The net percentage of radiolabel precipitated in the absence of inhibitor was 40% in one system and 55% in the other. In addition to the tests for idiotypic specificity described in Table I, each rabbit anti-idiotypic antiserum was assayed for its capacity to bind 0.01 μg of ^{125}I-labeled nonspecific IgG. Percentages of label precipitated varied from 6 to 9%.

[4] Pawlak, L. L., and A. Nisonoff. Unpublished data.

When necessary the specific removal of anti-Ar antibodies from mouse antisera was accomplished by using as the immunoadsorbent Sepharose 4B to which bovine gamma globulin (BGG)-*p*-azophenylarsonate was coupled by the cyanogen bromide technique (21). The weight of antigen coupled was approximately 5 mg/cm^3 of packed Sepharose. Control adsorptions were always run with unconjugated Sepharose.

TABLE I

Idiotypic Specificity of Absorbed Rabbit Antiserum Directed to Anti-Ar Antibody of Mouse 182

Unlabeled inhibitor	Specificity of inhibitors	Antibody content	Wt ratio unlabeled inhibitor to ^{125}I-labeled ligand	^{125}I-labeled ligand ppt.*
				% of control‡
Preimmune serum from donor mouse (10 μl)	Nonspecific	20–40 μg IgG (estimated)	2×10^3:4×10^3	98 ± 2
Hyperimmune donor serum	Anti-Ar	0.02 μg§	2:1	46 ± 6
		0.2 μg	20:1	0
IgG (A/J)	Nonspecific	2 mg	2×10^5:1	59 ± 2
Hyperimmune A/J serum‖	Anti-KLH	1 mg	10^5:1	63 ± 0
Hyperimmune A/J serum‖	Anti-KLH-*p*-azobenzoate	1 mg§	10^5:1	84 ± 1
BSA (2 mg)			2×10^5:1	93 ± 5
Hyperimmune donor¶ serum (adsorbed)		1 μl		82 ± 7
Control adsorption**		10 μg	10^3:1	0

* 40% of the labeled ligand (specifically purified anti-Ar antibody) was precipitated by the antiserum in the absence of inhibitor.

‡ ± average deviation.

§ Refers to content of precipitable antihapten antibody.

‖ Pooled hyperimmune serum from 10 mice other than donor.

¶ With Sepharose-BGG-Ar.

** With Sepharose.

RESULTS

Specificity of Absorbed Rabbit Antisera for Idiotypic Determinants.—Evidence has been presented elsewhere (14, 17) for the anti-idiotypic specificity of the absorbed rabbit antisera prepared against anti-Ar antibodies of mice 413 and 126. The data in Table I provide similar evidence for antibody directed to the anti-Ar antibody of mouse 182. Hyperimmune serum of the donor mouse strongly inhibited the binding of 0.01 μg of ^{125}I-labeled specifically purified antibody to its rabbit anti-idiotypic antibodies. An amount of unlabeled antiserum containing as little as 0.02 μg of precipitable anti-Ar antibody caused significant inhibition. In contrast, 10 μl of preimmune serum (containing about 20–40 μg of IgG) had no inhibitory effect. Adsorption of the hyperimmune serum with Sepharose-BGG-Ar to remove antihapten antibody removed all inhibitory capacity, whereas treatment with the same amount of unconju-

gated Sepharose had no significant effect. Pooled hyperimmune anti-KLH or anti-KLH-*p*-azobenzoate antisera from A/J mice caused only 37 and 16% inhibition when tested at the level of 1 mg of precipitable antibody, i.e., with a ratio of unlabeled ligand of 10^5:1. Two mg of IgG of the A/J strain caused 41% inhibition; bovine serum albumin (BSA) was noninhibitory. Thus, on a weight basis the unlabeled anti-Ar antibody of the donor mouse, no. 182, was more than 5×10^4 times as effective as an inhibitor as each of the other immunoglobulins or BSA. The results indicate that the absorbed rabbit antiserum recognizes idiotypic determinants. Similar quantitative results have been obtained with other anti-idiotypic antisera (17, 22).

An additional test for idiotypic specificity was carried out with the antiserum against anti-Ar antibodies from mouse 126. Equal volumes of preimmune serum from each of 10 mice of each inbred strain investigated (see Tables III and IV) were pooled. 50 μl of the pooled serum was then tested as an inhibitor in the indirect assay system. The percentage of labeled ligand bound varied from 81 to 110% of the control value. The amount of IgG in 50 μl is roughly 100–200 μg; nevertheless, the maximum degree of inhibition was much lower than that caused by 0.02 μg of the unlabeled donor antibody. This indicates that the cross-reactive idiotype is not present in appreciable concentration in preimmune serum of any of the strains of mice investigated.

A number of additional tests were carried out by immunoadsorption to ascertain whether the inhibitory capacity resides uniformly in the anti-Ar population. Antisera from three mice of each strain which showed significant inhibitory capacity (A/J, A/He, AL/N, A/WySn, A.By/Sn, A.Sw/Sn) and antisera from all of the BC9 mice were treated individually with the Sepharose-BGG-Ar adsorbent. In every instance the anti-Ar antiserum was no longer effective as an inhibitor after the immunoadsorption; the maximum degree of inhibition by 20 μl of adsorbed serum was 17%.

Intrastrain Idiotypic Cross-Reactions of A/J Anti-Ar Antibodies.—The data in Table II summarize the reactions of anti-Ar antibodies from individual, hyperimmune A/J mice with three anti-idiotypic antisera, prepared against specifically purified anti-Ar antibodies of mice 126, 182, and 413. Reactivity of Anti-Ar antibodies from different mice with anti-idiotypic antisera was quantified by measurement of the capacity of each hyperimmune antiserum to inhibit the binding of 0.01 μg of ^{125}I-labeled anti-Ar antibody of the donor mouse to its anti-idiotypic antibodies. Inhibitory capacity is expressed in terms of the amount of unlabeled precipitating anti-Ar antibody necessary to cause 50% inhibition of binding. This value is obtained by interpolation on a curve of percent inhibition vs. amount of unlabeled inhibiting antibody present. The experimental error in the interpolated value is estimated to be of the order of 20%.

Individual antisera were tested as inhibitors of two, and in some cases all three, of the anti-idiotypic antisera (Table II). For this reason each antiserum

TABLE II

Intrastrain Idiotypic Cross-Reactions of A/J Anti-Ar Antibodies

System	Donor of anti-Ar antibody (mouse no.)	Wt of A/J anti-Ar antibody needed for 50% inhibition of binding*	Median value
		μg	μg
A	126	$(0.02)_a$, 0.02_b, 0.02_c, 0.025_d, 0.03_e, 0.04_f, 0.05_g, 0.05_h, 0.05_i, 0.1_j, 0.15_k, 0.2_l, 0.2_m, 0.3_n, 0.3_o, 0.35_p, 0.4_q, 0.45_r, 0.5_s, 0.5_t, 0.5_u	0.15
B	182	$(0.02)_g$, $0.05_{a,i,o}$, 0.08_f, $0.15_{e,h}$, $0.2_{m,s}$, $0.3_{j,k,l,\ p,q,t}$, 0.35_c, $0.4_{d,u}$, $0.45_{n,r}$, 0.6_b	0.30
C	413	$(0.05)_f$, 0.06_g, 0.1_a, 0.2_b, $0.3_{c,d,h,i}$, 0.5_e, 0.6_k, 1.3_r, 1.9_l	0.30

* 0.01 μg of ^{125}I-labeled, specifically purified anti-Ar antibody of the donor mouse was used in the indirect precipitation test (see Methods). Whole sera were tested as inhibitors; data are expressed as weights of precipitable anti-Ar antibody required for 50% inhibition. Each value represents an individual A/J mouse, which is designated by a letter of the alphabet shown as a subscript to numerical values in the table. Unlabeled antiserum from each donor mouse was also tested; these values are given in parentheses.

is identified by a letter of the alphabet, included as a subscript after the corresponding experimental value in Table II. The value for unlabeled anti-Ar antibodies of the donor mouse in each system is given first and in parentheses.

Anti-Ar antibody from each mouse tested was a potent inhibitor of each of the three anti-idiotypic antibody preparations (Table II), although considerable variation is seen in the amounts of unlabeled antibody required for 50% inhibition. However, even the highest values (0.5–1.9 μg) are extremely small when compared with the milligram quantities of unrelated antibodies which fail to give 50% inhibition (Table I). In nearly all cases unlabeled antibodies from mice other than the donor were less effective as inhibitors than the unlabeled donor antibodies.

By noting the letter subscript associated with each value one can ascertain whether the antibodies of the various mice fall in the same order of effectiveness with each of the three sera tested (although not all of the group was tested in system C, Table II). It is apparent that there is some correlation in the order of effectiveness but also marked deviations, e.g. mice b, c, and d, whose antibodies are very effective in system A but relatively less effective in system B. Antisera from mice j, k, l, n, q, r, t, and u were among the less effective inhibitors in each system in which they were tested. Antibody from mouse a was a potent inhibitor in each system. In the three systems studied the median values for all mice (last column Table II) are 7.5, 15, and 6 times as great, respectively, as the value for the amount of unlabeled donor antibody required for 50% inhibition.

Strains of Mice Bearing the Cross-Reactive Anti-Ar Idiotype.—The strains of

mice studied clearly fell into two groups in terms of the idiotype of their anti-Ar antibodies. One group synthesized anti-Ar antibodies bearing the cross-reactive idiotype (CRI) in concentrations comparable to those found in the A/J strain. In the other group the amounts of CRI produced were far smaller. There is a clear distinction between the groups and we have so far not discovered strains producing intermediate concentrations of CRI.

Data on strains, in addition to A/J, which did synthesize idiotypically cross-reacting antibodies are shown in Table III. These strains are A/He, AL/N, and A/WySn; two congenic strains (A.By/Sn, A.Sw/Sn) in which the histocompatibility genes of the indicated strain have been introduced into a strain A background; and the BC9 congenic strain, bearing the AL/N allotype on a BALB/c background.

The values in the fourth column of Table III show the weights of precipitable anti-Ar antibodies from individual mice of the specified strain that caused 50% inhibition of binding of the labeled donor antibody. The median values (fifth column) and range are quite similar for each of the pure and congenic strains.

TABLE III

Inhibition of Binding of ^{125}I-Labeled Anti-Ar Antibody of Mouse 126 to Its Anti-Idiotypic Antibody. Inhibitors: Anti-Ar Antisera from Mice of Strains Producing the Cross-Reacting Idiotype

Strain	Heavy chain linkage group* (allotype)	*H-2* type	Wt of antibody required for 50% inhibition of binding‡	Median value
			μg	μg
A/He	a^4	a	0.05, 0.05, 0.06, 0.08, 0.08, 0.1, 0.1, 0.1, 0.1, 0.1, 0.2, 0.3, 0.4	0.10
AL/N	$a^{(4)}$§	a	0.03, 0.04, 0.05, 0.05, 0.08, 0.08, 0.08, 0.1, 0.4, 0.4, 0.5, 0.8, 1.1, 1.2	0.09
A/WySn	a^4	a	0.05, 0.08, 0.1, 0.1, 0.15, 0.2, 0.4, 0.4, 0.5, 0.9	0.17
A.Sw/Sn (congenic)	a^4	s	0.05, 0.05, 0.05, 0.05, 0.05, 0.1, 0.15, 0.15, 0.2, 0.25, 0.25, 0.3, 0.3, 0.35, 0.35, 0.35, 0.4	0.17
A.By/Sn (congenic)	a^4	b	0.1, 0.15, 0.15, 0.3, 0.35, 0.45, 0.5	0.30
BC9 (congenic)	$a^{(4)}$§	d	0.05, 0.2, 0.25, 0.35, 0.4, 0.45, 0.5, 0.5	0.37

* The abbreviations used for the heavy chain linkage groups are taken from Lieberman and Humphrey (24).

‡ See Table II, first two sentences of footnote *. The weight of unlabeled antibody from the autologous (donor) mouse required for 50% inhibition was 0.02 μg.

§ The immunoglobulin of the AL/N strain has one additional determinant, 18, which is not assigned to a particular immunoglobulin class. A separate designation for the linkage groups of the AL/N strain was not assigned by Lieberman and Humphrey (24). It is placed in a separate group by Herzenberg et al. (27).

The median value for the BC9 strain, 0.36 μg, is slightly more than twice as great as that for isologous A/J mice (Table II). This difference, although small, is probably significant since more than 0.2 μg of antibody was required, with seven of the eight BC9 antisera tested, whereas less than 0.2 μg of A/J antibody is required for 50% inhibition in most instances. We consider other differences among the strains listed in Table III to be of marginal significance. In addition, with the possible exception of the BC9 group, mice of each strain produced the cross-reactive idiotype in a concentration, on the average, comparable to that of the A/J strain (to which the donor mouse belonged). Also, within each strain there is a considerable spread of values for individual mice but in no instance was more than 1 μg of unlabeled antibody required for 50% inhibition of binding. Genetic relationships among these strains of mice will be considered in the Discussion.

Table IV presents inhibition data on anti-Ar antibodies from strains of mice which produced little or no antibody with the cross-reactive idiotype. A single, relatively large amount of unlabeled antibody (10 μg) was tested for its inhibitory capacity. From most strains listed in Table IV, none of the mice pro-

TABLE IV

Inhibition of Binding of ^{125}I-Labeled Anti-Ar Antibody of Mouse 126 to Its Anti-Idiotypic Antibody by Anti-Ar Antisera from Strains Producing Little Cross-Reactive Idiotype

Strain	Heavy chain linkage group (allotype)	*H-2* type	^{125}I-labeled ligand bound in presence of inhibitory antiserum*	Median value
			% of control‡	%
BALB/c	a^1	d	63, 72, 85, 89, 92, 98, 99, 99, 102, 103	95
CBA	a^1	k	97, 97, 97, 97, 98, 98, 99, 99, 100, 102	98
C3H	a^1	k	76, 89, 89, 95, 96, 96, 97, 97, 99	96
C57BR/cdJ	a^1	k	65, 66, 68, 76, 79, 88, 90, 92, 92, 96	84
C57BL/6J	a^2	b	41§, 46§, 51, 53, 60, 76, 77, 85, 87, 91	68
LP/J	a^2	b	95, 96, 97, 97, 98, 98, 99, 100, 100, 100	98
SJL/J	a^2	s	75, 88, 92, 92, 95, 95, 96, 96	94
SM/J	a^2	v	67, 67, 70, 72, 79, 79, 85, 94, 95, 101	79
B10.A	a^2	a	24‖, 35‖, 45‖, 58, 64, 67, 68, 73, 83, 85	66
DBA/2J	a^3	d	35¶, 40¶, 43¶, 53, 53, 54, 55, 65, 66, 72, 73, 73, 76, 77	60
RF/J	a^3	k	65, 71, 72, 72, 73, 76, 77, 77, 78, 106	75
SWR/J	a^3	b	64, 91, 92, 95, 96, 97, 98, 99, 103, 106	97
NZB	a^4	d	85, 91, 92, 94, 95, 98, 99, 99, 100, 101	97
AKR/J	$a^{(4)}$	k	86, 87, 94, 95, 95, 96, 96, 97, 99, 105	96

* Each serum contained 10 μg of precipitating anti-Ar antibody. The average deviation from the mean, expressed as percent of control, was 4%.

‡ Each value is an average of a duplicate determination.

§ 6 and 6.5 μg of anti-Ar antibody required for 50% inhibition.

‖ 2, 3, and 5.5 μg of anti-Ar antibody required for 50% inhibition.

¶ 6–6.5 μg of anti-Ar antibody required for 50% inhibition.

duced antibodies that caused 50% inhibition of binding when tested at the 10 μg level. The only exceptions were the DBA and C57BL strains and the B10.A congenic mice, carrying the *H-2* type of strain A on a C57BL background. Even in these strains the antisera of most mice failed to cause 50% inhibition; and for those antisera which did the weights of antibody needed were high as compared with the strains listed in Table III. Many of the strains produced antibodies that appear almost completely non-cross-reactive, as indicated by the median values in the last column of Table IV.

The above results were confirmed with another anti-idiotypic antiserum, directed to the anti-Ar antibody of mouse 182 (Table V). In this set of experiments, each anti-Ar antiserum was tested as inhibitor at a single concentration, using an amount of antiserum which contained 1 μg of precipitable anti-Ar antibody.

TABLE V

Inhibition of Binding of 0.1 μg of ^{125}I-Labeled Anti-Ar Antibody of Mouse 182 to Its Anti-Idiotypic Antibodies by Anti-Ar Antisera from Mice of Strains Other than A/J

Strain	Heavy chain linkage group (allotype)	*H-2* type	No. of mice tested	^{125}I-labeled ligand bound in presence of inhibitory antiserum*	
				Range	Median value
				% of control‡	
A/He	a^4	a	10	34–47	41
AL/N	$a^{(4)}$§	a	10	36–56	39
A/WySn	a^4	a	10	35–46	43
A.Sw/Sn (congenic)	a^4	s	10	34–52	43
A.By/Sn (congenic)	a^4	b	7	43–53	48
BC9 (congenic)	$a^{(4)}$§	d	5	43–60	49
BALB/c	a^1	d	10	85–103	95
CBA	a^1	k	10	101–111	106
C3H/J	a^1	k	9	92–105	102
C57BR/cdJ	a^1	k	10	80–108	96
C57BL/6J	a^2	b	10	72–103	86
LP/J	a^2	b	10	94–110	101
SJL/J	a^2	s	8	93–107	99
SM/J	a^2	v	10	79–104	90
B10.A (congenic)	a^2	a	10	50–98	90
DBA/2J	a^3	d	10	72–99	87
RF/J	a^3	k	10	82–93	89
SWR/J	a^3	b	10	88–97	93
NZB	a^4	d	10	93–104	101
AKR/J	$a^{(4)}$	k	10	93–103	99

* The amount of each serum tested contained 1 μg of precipitating anti-Ar antibody.

‡ Each value is an average of a duplicate determination. The overall average deviation from the mean, expressed as percent of control, was 5.7%.

§ See footnote §, Table III.

The results are virtually the same as those obtained with the other anti-idiotypic antiserum. Anti-Ar antisera from each of the A strains and the congenic strains A.By/Sn and A.Sw/Sn were inhibitory. Anti-Ar antibody of the BC9 strain, bearing the allotype marker of AL/N on a BALB/c background, was also a strong inhibitor. All other strains produced anti-Ar antibodies that were noninhibitory or that gave a marginal degree of inhibition.

Quantitative Inhibitory Capacity of Antibodies from F_1 Mice.—Fig. 1 shows data on the interaction with anti-idiotypic antiserum of anti-Ar antibodies prepared in two F_1 strains, C57BL × A/J and BALB/c × A/J. The data are presented as the weight of unlabeled precipitable anti-Ar antibody required for 50% inhibition of binding. Although nearly all antibody preparations are capable of inhibiting the anti-idiotypic antibodies, the individual sera vary

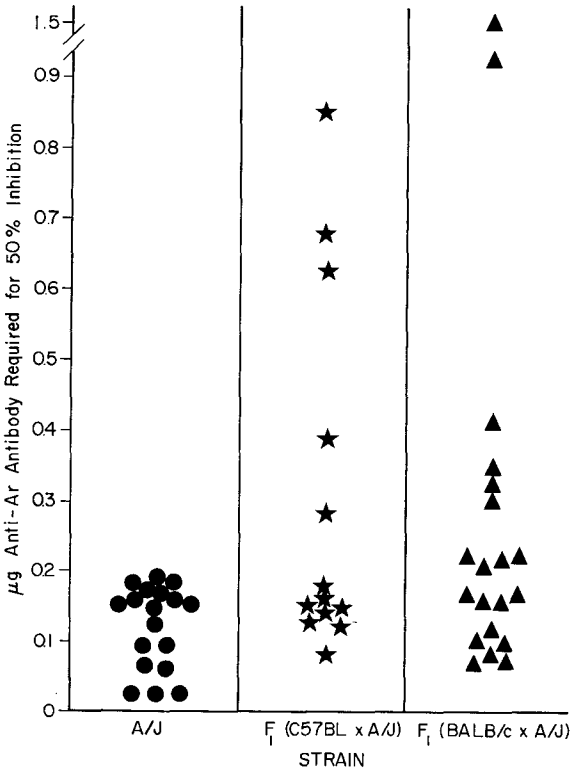

FIG. 1. Amounts of unlabeled, precipitable anti-Ar antibody from individual mice required for 50% inhibition of binding of 0.01 μg of ^{125}I-labeled anti-Ar antibody of A/J mouse 126 to its rabbit anti-idiotypic antibodies. Each point represents an individual mouse. Antibodies from all mice of the A/J strain caused more than 50% inhibition. Antisera from 3 F_1 (C57BL × A/J) and 1 F_1 (BALB/c × A/J) mouse, each containing 2 μg of precipitable antibody, failed to cause 50% inhibition.

markedly on a quantitative basis; many are equally as potent, on a weight basis, as A/J antibodies, whereas others are relatively ineffective, although significant inhibitory capacity is observed in nearly all preparations when tested at levels of 1–2 μg. 3 of 16 C57BL × A/J and 1 of 21 BALB/c × A/J mice produced antibodies that caused less than 30% inhibition when 2 μg was tested.

DISCUSSION

It is possible to consider linkage of idiotype to allotype or to *H-2* type at two levels. One is the linkage observed in laboratory studies, for example in congenic mice; the other is any apparent linkage noted in different inbred strains. Before discussing the present data we will review briefly experiments which have been carried out with congenic or recombinant-inbred mice and which bear on linkage of idiotype to allotype.

In the case of the anti-Ar idiotype very close linkage to heavy chain allotype was observed in all mice of the BC9 strain, which have the heavy chain allotype of the AL/N strain on a BALB/c background (17–19). The gene(s) controlling anti-Ar idiotype must therefore be closely linked to genes encoding C_H regions of mouse immunoglobulin polypeptide chains. Similarly, linkage between allotype and idiotype was demonstrated by Blomberg et al. (15) in recombinant-inbred strains. Their results with congenic mice were, however, not consistent with close linkage and were explained on the basis of a crossover. Sher and Cohn (23) found that two congenic mice bearing the heavy chain allotype of the AL/N strain on a BALB/c background produced anti-PC antibodies, some of which were not reactive with an anti-idiotypic antiserum which recognizes essentially all anti-PC antibodies produced by normal BALB/c mice.[5] This finding would indicate either that there is close linkage between AL/N allotype and the idiotype expressed, i.e. that the non-cross-reacting idiotype is characteristic of AL/N mice, or, conceivably, that the congenic strain produces anti-PC antibodies only of BALB/c origin, some of which possess other non-cross-reacting anti-PC idiotypes, which are sometimes observed in BALB/c mice (12).

The present investigation was concerned with the association of idiotype with allotype or *H-2* type among different inbred strains and in congenic mice. It is evident, first, that there is no linkage of the anti-Ar idiotype to *H-2* histocompatibility type. This is shown by results obtained with the congenic B10.A strain. Despite the fact that these mice have the *H-2* type of the A strain (on a

[5] Linkage of the anti-Ar idiotype to allotype was shown by the presence of the characteristic AL/N idiotype in congenic mice bearing the AL/N allotype on a BALB/c background. Linkage of the BALB/c anti-PC idiotype to allotype was based on the absence of the idiotype from some anti-PC molecules in congenic mice having an unrelated allotype on a BALB/c background.

C57BL background), their anti-Ar antibodies do not exhibit the CRI. Conversely, congenic mice bearing the *H-2* type of other strains on a strain A background (A.Sw/Sn, A.By/Sn) produced concentrations of CRI comparable to that of the A/J strain. As previously reported (19), anti-Ar antibodies of the BC9 strain, possessing the AL/N allotype on a BALB/c background, all were strongly inhibitory.

Somewhat different results were reported by Sher and Cohn (23). In BALB/c mice, immunized with pneumococcal C-carbohydrate conjugated to sheep erythrocytes (SRBC), the formation of anti-PC plaques is entirely inhibitable by an anti-idiotypic (anti-S107) antiserum; in strain A/WySn, on the other hand, plaque formation is only slightly or not at all inhibitable. However, the congenic strain, A.By/Sn, bearing the *H-2* type of strain BY on an A/WySn background, forms plaques that are inhibitable at an intermediate level. This was interpreted as suggesting that the *H-2* gene of the BY strain is linked to a gene controlling synthesis of an immunoglobulin polypeptide, possibly V_κ, of the BY strain, which bears idiotypic determinants recognized by anti-S107.[6]

We would suggest the alternative possibility that the idiotype expressed could depend on the nature of the thymus-derived (T) cell membrane of the A.By/Sn mice, which in turn is influenced by the *H-2* gene. A given type of T cell might favor the expression of particular bone marrow-derived (B) cells, i.e. of particular idiotypes, either through its mode of presentation of antigen to B cells or by humoral factors released. One could then postulate that the A/WySn strain itself possesses B cells bearing receptors with the S107 idiotype, but that these are normally not expressed or are expressed at a low level as compared with other anti-PC idiotypes. Alterations of the T cell (or conceivably of the B cell) membrane, as in the congenic A.By/Sn strain, might permit some expression of the S107 idiotype. Some support for this possibility is derived from the results obtained by Sher and Cohn (23) with F_1 (BALB/c $\times$ A/J) mice. Since A/J mice synthesize anti-PC antibodies which entirely lack the S107 idiotype, the F_1 mice must also have the genetic capability of producing non-cross-reactive as well as cross-reactive anti-PC antibodies. But in fact nearly all anti-PC antibodies synthesized by the F_1 mice carried the S107 idiotype. Thus, the expression of the BALB/c idiotype in these mice was favored at the expense of the A/J idiotype. This demonstrates that the genetic capability of producing an idiotype is not equivalent to its expression. It seems conceivable then that the A/WySn strain has such genetic information, which is not expressed in the pure strain, but is reflected in the congenic mice, either through the mechanism suggested above or through some other mechanism relatable to *H-2* type. Such an alternative might eliminate the necessity of postulating linkage of V_κ to *H-2*.

[6] This explanation assumes that the BY strain produces the cross-reactive idiotype. The BY strain itself cannot be tested since it is no longer available.

The question of linkage with respect to allotype in the different strains of mice we studied is less clear. All strains which exhibited CRI, with the exception of the closely related AL/N strain (see Methods), belong to the a^4 heavy chain linkage group of Lieberman and Humphrey (24). However, NZB mice, which are in the same linkage group, failed to produce CRI.

Several explanations for these results may be considered. A crossover between the gene(s) controlling CRI and the IgC_H region may have occurred in an ancestor of the NZB strain. A second possibility is that the A and NZB strains had a common ancestor, that divergence occurred long before the inbreeding process was undertaken,[7] and that the *V* genes have undergone evolution since that time. The fact that the allotype has remained the same could reflect a slower rate of evolution of C_H genes or the failure of antisera to detect those changes in amino acid sequence which may have occurred in the C_H region. The fact that NZB mice are of a different *H-2* type than the A strain is probably not directly relevant since congenic mice of different *H-2* types on an A strain background produce CRI. However, the difference in *H-2* type emphasizes the fact that NZB and A mice may not be very closely related, despite their shared allotype.

In a similar context, the AKR strain, which has the same heavy chain allotype as AL/N (an allotype closely related to that of A/J mice), differs from AL/N in that it fails to produce CRI. The AL/N strain is thought to have originated from an outcrossed strain A mouse, whereas the origin of the AKR strain appears unrelated to that of A (25). The fact that the AL/N strain, but not AKR, has the same *H-2* type as A/J is probably relevant only insofar as it may reflect a common origin of the AL/N and A/J strains. Actually, our data would tend to support the conjecture (25) that the AL/N strain has a strain A ancestor, since other strains producing CRI are all closely related to strain A.

In addition to the AL/N strain, to the BC9 strain which possesses the AL/N allotype, and to the congenic mice with a strain A background, the strains producing CRI are A/He and A/WySn. Both are of the same allotype and *H-2* type as A/J and both are sublines of the A strain (25).

One interpretation of these patterns of cross-reactivity is that different strains of mice have different sets of germ line *V* genes. An alternative possibility is that different strains have similar germ line genes but that a regulatory gene controls the production of CRI. If so, this gene must be closely linked to the IgC_H locus, as evidenced by the production of CRI by the BC9 mice.

Of the two interpretations, we would favor as the simplest possibility the view that synthesis of CRI is based on the presence of appropriate genes controlling V regions of immunoglobulins, and that CRI therefore is a genetic marker for the V_H region. Its close linkage to the IgC_H locus (17–19) is compatible with this possibility.

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[7] It is relevant that the NZB mice were inbred in England, whereas the A strain was inbred in the United States.

A striking feature of the results is the clear separation among strains with respect to ability to produce CRI. On a quantitative basis all nonproducing strains synthesize less than 1/10 as much of the CRI as those exhibiting the idiotype; the actual ratios are probably much smaller. It is evident then that each positive strain synthesizes CRI in substantial quantities. Since we know that the A/J strain is capable of producing anti-Ar antibodies with a great variety of idiotypes (26), the preferential biosynthesis of CRI may reflect its association with an immunoglobulin of relatively high affinity which, as a lymphocyte surface receptor, competes effectively for antigen. The question of affinity is being investigated.

The studies with F_1 mice demonstrate that, with very few exceptions, nearly all such mice (BALB/c × A/J or C57BL × A/J) produce anti-Ar antibodies with CRI. However, the proportion of the anti-Ar population carrying the idiotype varies considerably among individuals. Antibodies from many F_1 mice are as effective, on a weight basis, as those of the average A/J mouse; a few mice, however, synthesized considerably less CRI. On the average, the F_1 mice clearly produced more than half as much CRI as the parent A/J mice (Fig. 1). Two alternative interpretations of these results are: (*a*) The proportion of lymphocytes bearing anti-Ar receptors characteristic of the A/J strain varies among individual F_1 mice, but there is a tendency for such lymphocytes to predominate; (*b*) the numbers of lymphocytes carrying receptors characteristic of A/J or the non-A/J strain are comparable, but the A/J receptors are on the average of greater affinity. Which idiotypes are stimulated in an individual mouse would, in this case, be partly a matter of chance, with higher affinity receptors tending to favor the A/J idiotype. Once established, any set of clones might be difficult to replace because memory cells, present at high concentration, would tend to compete effectively for the antigen.

The results suggest that the failure of BALB/c or C57BL mice to express the cross-reactive anti-Ar idiotype is attributable to a difference, as compared with A/J, in germ line *V* genes rather than to the presence of a regulatory gene which prevents the expression of the cross-reactive anti-Ar idiotype. Such a gene should have been present in the F_1 mice and suppressed the appearance of the idiotype.

While the presence of certain idiotypes in some strains of mice but not in others is obviously consistent with the presence of large numbers of *V* genes, which differ among strains (theory of multiple germ line genes), it can also be interpreted via somatic mutation. If a gene which encodes a polypeptide chain of a particular antihapten antibody can be derived from a germ line gene by a small number of mutations it might occur in nearly all mice of a given strain, despite the necessity for random mutation to arrive at the required DNA sequence. Mice of another strain, possessing different germ line genes, might, through mutation, attain an appropriate but different nucleotide sequence by a similar mechanism, thus accounting for the observed variations in idiotype of anti-Ar antibodies among strains.

SUMMARY

The expression of an idiotype characteristic of anti-*p*-azophenylarsonate antibodies of all A/J mice was explored in F_1 progeny, in other inbred strains, and in congenic mice. Of the strains tested only those closely related to A/J produced antibodies with the cross-reactive idiotype (CRI). None of the mice synthesized intermediate levels of CRI. No relationship between *H-2* type and idiotype was noted. Congenic mice with a strain A background but a different *H-2* type produced CRI in amounts quantitatively equivalent to those of strain A mice. Conversely, the presence of the *H-2* genotype of strain A on an unrelated background was not associated with the formation of CRI. Nearly all F_1 progeny of strain A mice formed CRI, indicating that failure of the other (non-A) parental strain to produce CRI is not attributable to the presence of a gene controlling the synthesis of a suppressor of CRI. NZB mice, which have the same heavy chain allotype as strain A, but are unrelated in origin, failed to produce CRI, although allotype has been shown to be linked to idiotype in congenic strains.

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REFERENCES

1. Slater, R. J., S. M. Ward, and H. G. Kunkel. 1955. Immunologic relation among the myeloma proteins. *J. Exp. Med.* **101**:85.
2. Oudin, J., and M. Michel. 1963. Une nouvelle forme d'allotypie des globulines y du sérum de lapin apparemment liée à la jonction et à la specificité anticorps. *C. R. Seances Acad. Agric. Fr.* **257**:805.
3. Kunkel, H. G., M. Mannik, and R. C. Williams. 1963. Individual antigenic specificities of isolated antibodies. *Science (Wash. D. C.)*. **140**:1218.
4. Kunkel, H. G. 1970. Individual antigenic specificity, cross specificity, and diversity of human antibodies. *Fed. Proc.* **29**:55.
5. Wilson, S. K., B. W. Brient, and A. Nisonoff. 1971. Individually specific antigenic determinants shared by a myeloma protein and nonspecific IgG. *Ann. N. Y. Acad. Sci.* **190**:364.
6. Grey, H. M., M. Mannik, and H. G. Kunkel. 1965. Individual antigenic specificity of myeloma proteins. Characteristics and localization of subunits. *J. Exp. Med.* **121**:561.
7. Brient, B. W., and A. Nisonoff. 1970. Quantitative investigations of idiotypic antibodies. IV. Inhibition by specific haptens of the reaction of anti-hapten antibody with its anti-idiotypic antibody. *J. Exp. Med.* **132**:951.
8. Brient, B. W., J. Haimovich, and A. Nisonoff. 1971. Reaction of antiidiotypic antibody with the hapten-binding site of a myeloma protein. *Proc. Natl. Acad. Sci. U. S. A.* **68**:3136.
9. Sirisinha, S., and H. Eisen. 1971. Autoimmune-like antibodies to the ligand-binding sites of myeloma proteins. *Proc. Natl. Acad. Sci. U. S. A.* **68**:3130.
10. Sher, A., and M. Cohn. 1972. Effect of haptens on the reactions of antiidiotype

antibody with a mouse antiphosphorylcholine plasmacytoma protein. *J. Immunol.* **109:**176.

11. Hopper, J. E., and A. Nisonoff. 1971. Individual antigenic specificity of immunoglobulins. *Adv. Immunol.* **13:**57.
12. Potter, M., and R. Lieberman. 1970. Common individual antigenic determinants in five of eight BALB/c IgA myeloma proteins that bind phosphorylcholine. *J. Exp. Med.* **132:**737.
13. Cosenza, H., and H. Kohler. 1972. Specific inhibition of plaque formation to phosphorylcholine by antibody against antibody. *Science* (*Wash. D. C.*). **176:**1027.
14. Kuettner, M. K., A. L. Wang, and A. Nisonoff. 1972. Quantitative investigations of idiotypic antibodies. VI. Idiotypic specificity as a potential genetic marker for the variable regions of mouse immunoglobulin polypeptide chains. *J. Exp. Med.* **135:**579.
15. Blomberg, B., W. R. Geckeler, and M. Weigert. 1972. Genetics of the antibody response to dextran in mice. *Science* (*Wash. D. C.*). **177:**178.
16. Eichmann, E. 1972. Idiotypic identity of antibodies to streptococcal carbohydrate in inbred mice. *Eur. J. Immunol.* **2:**301.
17. Pawlak, L. L., D. A. Hart, A. Nisonoff, E. B. Mushinski, and M. Potter. 1973. Idiotypic specificity and the biosynthesis of antibodies. Proceedings of 3rd International Convocation of Immunology. In press.
18. Nisonoff, A. 1972. *In* Genetic Control of Immune Responsiveness. Proceedings of Brook Lodge Symposium, May, 1972. M. Landy and H. O. McDevitt, editors. Academic Press, Inc., New York.
19. Pawlak, L. L., E. B. Mushinski, A. Nisonoff, and M. Potter. 1973. Evidence for the linkage of the IgC_H locus to a gene controlling the idiotypic specificity of anti-*p*-azophenylarsonate antibodies in strain A mice. *J. Exp. Med.* **137:**22.
20. Sommerville, R. G. 1967. The production of fluorescent antibody reagents for virus diagnosis in the albino mouse. *Arch. Virusforsch.* **20:**445.
21. Axen, R., J. Porath, and S. Ernback. 1969. Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature* (*Lond.*). **214:**1302.
22. Pawlak, L. L., A. L. Wang, and A. Nisonoff. 1972. Concentration of cross-reacting idiotypic specificities in unrelated mouse immunoglobulins. *J. Immunol.* In press.
23. Sher, A., and M. Cohn. 1972. Inheritance of an idiotype associated with the immune response of inbred mice to phosphorylcholine. *Eur. J. Immunol.* **2:**319.
24. Lieberman, R., and W. Humphrey, Jr. 1971. Association of H-2 types with genetic control of immune responsiveness to IgA allotypes in the mouse. *Proc. Natl. Acad. Sci. U. S. A.* **68:**2510.
25. Staats, J. 1972. Standardized nomenclatures for inbred strains of mice. Fifth listing. *Cancer Res.* **32:**1609.
26. Hart, D. A., L. L. Pawlak, and A. Nisonoff. 1972. Nature of antihapten antibodies arising after immune suppression of a set of cross-reactive idiotypic specificities. *Eur. J. Immunol.* In press.
27. Herzenberg, L. A., H. O. McDevitt, L. A. Herzenberg. 1968. Genetics of antibodies. *Annu. Rev. Genet.* **2:**209.