IDIOTYPE EXPRESSION AND THE INHERITANCE OF MOUSE ANTIBODY CLONES*

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A number of recent reports from several laboratories have indicated that idiotypes (1–3) of certain antibodies behave as genetic markers. Such idiotypes are common among the antibodies with the same specificity in closely bred rabbits (4) and in inbred mice (5–9) and are transmitted from parents to offspring in breeding experiments (4, 8, 9). Several of these observations employed antibodies to the group-specific cell wall carbohydrates of streptococci (4, 5, 9). The restricted heterogeneity of these antibodies has been extensively documented in rabbits (10) and mice (5, 11).

The present investigation employed antibodies to the group-specific carbohydrates of Group A and Group C streptococci, raised in inbred mice. Experiments were performed to study the inheritance of single mouse antibody clones, using the idiotype as a marker for the variable (V)[1] region and the allotype as a marker for the constant (C) region of the clonal heavy chain. The evidence suggests that antisera against the idiotype of such clones can be successfully used to identify the clonal product in the antibodies of syngeneic, allogeneic, and hybrid mice. The results are indicative of a linked genetic transmission of the genes coding for the idiotype and the heavy chain C region allotype of an antibody clone. Furthermore, the strain specificity of idiotypes suggests that V genes exist in two or more allelic forms.

Materials and Methods

Mice.—Inbred strains A/J, BALB/cJ, CE/J, SWR/J, C57BL/6J, SJL/J, DBA/1J, DBA/2J, and C57L/J were obtained from the Jackson Laboratory, Bar Harbor, Maine. Colony-bred NMRI mice were purchased from Ivanovas, Kisslegg, W. Germany.

Streptococci, Group-Specific Carbohydrates, Streptococcal Vaccines, Immunization of Mice.— Group A, strain 17A4, and Group C, strain C74, streptococci were obtained from Dr. R. C. Lancefield, The Rockefeller University. Purified group-specific carbohydrates were a gift from Dr. R. M. Krause, The Rockefeller University. Streptococcal vaccines were prepared as de-

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* This work was supported by Sonderforschungsbereich 74.

[1] *Abbreviations used in this paper:* A-CHO, Group A streptococcal carbohydrate; anti-A, antibodies to A/J allotypes; anti-BALB, antibodies to BALB/cJ allotypes; anti-Fc, antibodies to mouse fragment Fc; BSA, bovine serum albumin; C, constant; C-CHO, Group C streptococcal carbohydrate; CHO, purified streptococcal carbohydrates; PBS, phosphate-buffered saline; RAG, rabbit antiserum to guinea pig IgG; V, variable.

scribed (10). Mice received two series of intravenous injections of diluted vaccine as previously described (5).

Cloning.—The in vivo cloning procedure of Askonas et al. (12) was adapted for mice immunized with streptococci, as described previously (5).

Isoelectric Focusing.—Isoelectric focusing employed the procedure of Awdeh et al. (13).

Radiolabeling.—Immunoglobulins were labeled with ^{125}I using the chloramine-T method (14). Purified streptococcal carbohydrates (CHO) were labeled with ^{125}I using the chloramine-T method after cyanogen bromide incorporation of tyramine, as previously described (4, 5).

Antigen-Binding Test.—The determination of the antibody concentration in antistreptococcal antisera employed a modified Farr assay (15) using [^{125}I]CHO as previously described (5).

Anti-Immunoglobulin Antisera.—The preparation of a sheep antiserum to mouse fragment Fc (anti-Fc) has been described (5). A rabbit antiserum to guinea pig IgG (RAG) was prepared by repeated intramuscular injection of pooled purified guinea pig IgG in complete Freund's adjuvant. All bleedings from three injected rabbits showing strong precipitating activity were pooled. In order to remove cross-reactivity with mouse IgG, this antiserum was adsorbed on Sepharose 2B-coupled mouse IgG (16).

Antiallotype Antisera.—A BALB/c anti-AL antiserum and a C57BL/6 anti-BALB/c antiserum were kindly provided by Dr. R. Lieberman, National Institutes of Health. The anti-BALB/c antiserum recognized the G$^{1, 6, 7, 8}$ allotypic determinants on the γ2a heavy chain constant region. The anti-AL antiserum recognized IgG heavy chain determinants in A/J mice not yet assigned to a specific class (R. Lieberman, personal communication).

Antisera to the Idiotype.—These were prepared in guinea pigs (a colony-bred albino line maintained in this laboratory) by previously described methods (4, 5). For the present investigation it was necessary to absorb the antisera with pooled anti-CHO antibodies in addition to the usual absorption with pooled mouse IgG (4).

Purification Procedures.—Mouse IgG was purified from pooled ascites developed in NMRI mice, from pooled normal BALB/cJ serum and from pooled normal A/J serum, using the method of Levy and Sober (17), modified as previously described (5). Purification of guinea pig IgG employed the original method of Levy and Sober (17).

Mouse antibodies to the streptococcal carbohydrates were purified by affinity chromatography, as previously described (18). Immunoadsorbents included Sepharose 4B conjugated with *p*-aminophenyl-*N*-acetyl-galactosamine for antibodies to Group C carbohydrate (C-CHO), and Sepharose 4B conjugated with *p*-aminophenyl-*N*-acetyl-glucosamine for antibodies to Group A carbohydrate (A-CHO).

Antiallotype antibodies were specifically purified by adsorbing the antiallotype antisera to glutaraldehyde cross-linked (19) normal serum of the homologous strain for 30 min under gentle rotation at room temperature and subsequent elution with 0.2 M glycine-HCl, pH 3.2, for 30 min at 0°C. The eluted antibodies were radiolabeled and their concentration was estimated by quantitative binding to the ethyl chloroformate cross-linked (20) anti-Fc serum. Approximately 60 μg of specific antiallotype antibody were recovered from 100 μl of each of the two antiallotype antisera. Antibodies to mouse Fc were purified from the anti-Fc serum in a similar fashion, except for the use of glutaraldehyde cross-linked NMRI serum as the immunoadsorbent in this case.

Idiotypic Binding and Inhibition Test.—For the quantitative determination of idiotypic binding using guinea pig anti-idiotypic antisera an indirect radioprecipitin test was employed (5, 21). The homologous radiolabeled mouse antibody was incubated with the anti-idiotypic antiserum, and the resulting complexes were precipitated by the rabbit antiguinea pig IgG antiserum (RAG). The binding capacity of each anti-idiotypic antiserum was determined using 20 ng of radiolabeled mouse antibody, 20 μl of serial dilutions of anti-idiotypic antiserum, and 20 μl of RAG. For most antisera, the end point of complete binding of 20 ng homologous antibody was at dilutions between 1:100 and 1:250.

At the predetermined end point of complete binding, cold immunoglobulins were added as

inhibitors. These included purified anti-CHO antibodies, normal mouse IgG, and whole anti-CHO antisera which were diluted to the appropriate antibody concentrations. All dilutions were done in phosphate-buffered saline, pH 6.8, containing 3% bovine serum albumin (PBS-BSA). The initial incubation period of [125I]mouse antibody, guinea pig antiserum, and inhibitor was 4 h at room temperature. This was followed by incubation overnight at 4°C with RAG. After centrifugation the amount of radioactivity in the precipitates was determined as described (5, 22).

Idiotype-Allotype Association Test.—For the investigation of the association between idiotype and allotype in heterozygous mice the following test system was developed: Anti-idiotypic antisera against a parental strain idiotype were insolubilized by ethylchloroformate (20) and homogenized. Such insoluble antisera were then incubated with anti-CHO antisera from F_1 hybrid mice, selectively adsorbing those antibodies possessing the parental strain idiotype. After extensive washing the sediments were incubated with specifically purified [125I]antiallotype antibodies, and, for control, [125I]anti-Fc antibodies. This allows the quantitative determination of the proportion of molecules possessing the allotype of each parental strain within the antibody population bound to the solid anti-idiotypic antiserum.

The detailed experimental procedure was as follows: Amounts of solidified anti-idiotypic antisera previously determined to bind 40 ng of antibody with the homologous idiotype were incubated with amounts of anti-CHO antisera containing 40 µg of antibody. After 4 h of gentle rotation at room temperature the sediments were washed four times with ice-cold PBS and resuspended in 400 µl of PBS-BSA. Three 100-µl aliquots of this suspension, each containing 10 ng of antibody bound to solid anti-idiotypic antiserum, were then transferred into three separate test tubes. 50 ng of [125I]antiallotype antibodies against each of the two parental strains and 100 ng of [125I]anti-Fc antibody were then added to the three test tubes, respectively. After another 4 h of gentle rotation at room temperature the amounts of radioactivity bound to the sediments were determined.

Because of the inaccuracy in pipetting suspensions, each experiment was performed in triplicate, and experiments that showed more than 10% deviation between the highest and the lowest value were repeated. It was, however, found that 3% BSA in the system decreased the spontaneous sedimentation so that the reproducibility was commonly better than ±5%.

RESULTS

In this paper, two series of experiments are presented which are concerned with two separate aspects of the inheritance of idiotypes. The first series of experiments was performed to obtain quantitative information on the expression of idiotypes. In these experiments, idiotypes were studied with respect to their frequency of expression in various inbred strains, and with respect to their proportions in the antibodies of individual mice. The second series of experiments was performed to study the genetic linkage of idiotypes and allotypes in heterozygous mice.

For this purpose the idiotypes of two monoclonal antibodies were studied. Both antibodies are produced by clones of spleen cells which were obtained from hyperimmunized A/J mice and maintained in vivo by serial spleen cell transfers (5, 12). Clone A5A is reactive with Group A streptococcal carbohydrate (A-CHO). Clone A2C is reactive with Group C streptococcal carbohydrate (C-CHO). The antibody preparations used here were isolated from the pooled sera of 22 immunized recipient mice bearing clone A5A, and from the pooled sera of 17 immunized recipient mice bearing clone A2C, respectively.

The isoelectric spectra of both antibodies are shown in Fig. 1. These spectra are developed by isoelectric focusing of the isolated antibodies in a pH 6–10 gradient. Antibody A5A focuses within three bands, indicating its monoclonal origin (5, 12). Antibody A2C focuses within five bands, leaving uncertainty about the number of clones involved in its production.

Idiotypic antisera to each of these isolated antibodies were raised in guinea pigs. All antisera were adsorbed to idiotypic specificity as described in the Methods section. Antisera to antibody A5A are referred to as anti-A5A; antisera to antibody A2C are referred to as anti-A2C. Except for the idiotype-allotype association test, all experiments were done with two or more antisera for each antibody. No qualitative differences were observed. Therefore, only the results obtained with one antiserum are reported below for each of the antibodies.

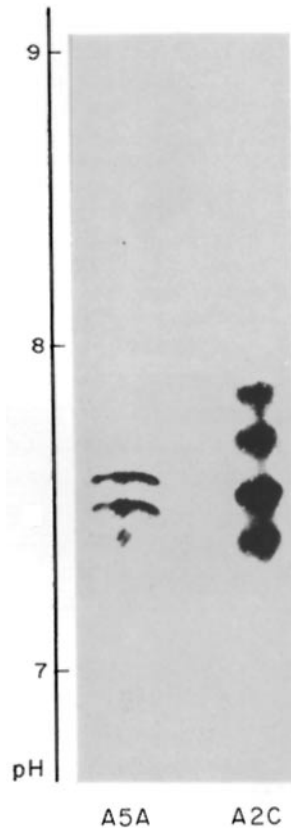Illustrated in Fig. 2 are inhibition experiments performed to test anti-A5A

FIG. 1. Isoelectric focusing spectra of isolated antibodies A5A and A2C. Gradient: pH 6–10. Coomassie blue stain. Due to loss of detail, a faint third band of antibody A5A and a faint fifth band of antibody A2C are not seen on the photograph.
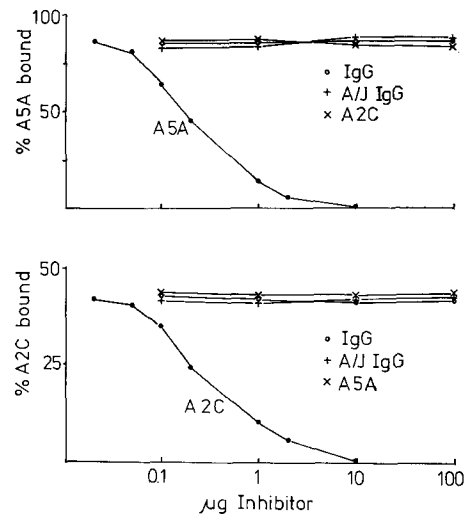
FIG. 2. Inhibition of idiotypic binding. Upper frame: inhibition of the idiotypic binding between 20 μl of anti-A5A, 1:250 dilution, and 20 ng of [^{125}I]antibody A5A. Inhibitors include cold antibody A5A (●—●), pooled mouse IgG (○—○), IgG from strain A/J (+—+), and antibody A2C (X—X). Lower frame: inhibition of the idiotypic binding between 20 μl of anti-A2C, 1:100 dilution, and 20 ng of [^{125}I]antibody A2C. The symbols designating the curves correspond to those indicated for the upper frame.

and anti-A2C for their idiotypic specificity. Inhibitions were performed at the predetermined end points of complete binding, which were reached at dilutions of 1:250 for anti-A5A and 1:100 for anti-A2C, using 20 μl of diluted anti-idiotypic antisera and 20 ng of radiolabeled homologous antibody. The top frame of Fig. 2 shows the inhibition of the idiotypic binding between radiolabeled antibody A5A and anti-A5A, obtained with 0.02–10 μg of cold antibody A5A, and with 0.1–100 μg of cold pooled IgG (IgG), A/J IgG, and antibody A2C, respectively. As was expected, antibody A5A inhibited the idiotypic binding, whereas nonspecific IgG and antibody A2C did not. Addition of pooled IgG in amounts greater than 100 μg caused some inhibition, which was partially due to inhibition of the precipitation between the guinea pig IgG and RAG. Therefore, amounts of inhibitor greater than 100 μg were not used in this test system. Similar results were obtained in the inhibition experiments performed with anti-A2C and radiolabeled antibody A2C, illustrated in the bottom frame of Fig. 2. None of the three heterologous inhibitors caused detectable inhibition in amounts of up to 100 μg. It is apparent from these inhibition experiments that both anti-A5A and anti-A2C are idiotypically specific antisera.

It should be mentioned that anti-A2C binds only 46% of the purified antibody A2C. This is consistent with the isoelectric focusing spectrum of antibody A2C (Fig. 1) which suggests an origin from more than one clone. Since only a fraction of antibody A2C is bound by anti-A2C, however, it is reasonable to assume that this antiserum detects a monoclonal idiotype as does anti-A5A.

The experiments in Fig. 2 clearly show the lack of idiotypic cross-specificity between antibodies A5A and A2C. Furthermore, experiments not reported here revealed that idiotypic cross-reactions were observed only among antibodies against the same carbohydrate antigen. Therefore, the experiments reported below were done with antibodies to A-CHO for the A5A idiotype and with antibodies to C-CHO for the A2C idiotype, respectively. For the sake of convenience, the results concerning idiotype expression and those concerning idiotype-allotype linkage are presented in separate sections.

The Expression of Idiotypes A5A and A2C in Inbred and Hybrid Mice.—In a previous study using isologous (A/J anti-A/J) anti-idiotypic antisera, it has been shown that the A5A idiotype is expressed in the antibodies to A-CHO from most A/J mice (5). The present experiments extend these observations using heterologous anti-idiotypic antisera, and testing a greater number of mice and strains. Furthermore, the system of inhibition of idiotypic binding was standardized to permit the quantitative determination of the proportions of antibody molecules possessing a given idiotype within the antibodies of each individual mouse.

This was done using artificial mixtures of cold homologous antibody and pooled IgG as inhibitors. The proportions of these mixtures were chosen to resemble those expected in the antibodies of most syngeneic mice. Fig. 3 shows the inhibition curves for both the A5A and A2C idiotypic binding systems obtained with pooled IgG, and pooled IgG containing 0.1, 1, and 10% of the
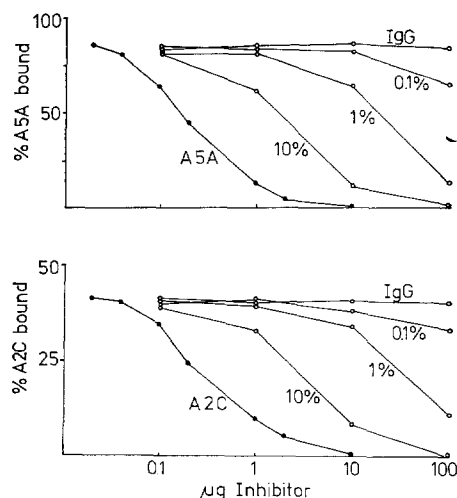

Fig. 3. Standardization of the inhibition of idiotypic binding using mixtures of cold pooled IgG with homologous antibody as inhibitors. The proportions of homologous antibody in the mixtures are given in the figure. Curves designated IgG, pooled IgG without homologous antibody. Upper frame: inhibition of the idiotypic binding between 20 μl of anti-A5A, 1:250 dilution, and 20 ng of [125I]antibody A5A. Lower frame: inhibition of the idiotypic binding between 20 μl of anti-A2C, 1:100 dilution, and 20 ng of [125I]antibody A2C.

homologous antibodies, respectively. It is apparent from the figure that the slopes of the curves for the various immunoglobulin mixtures are parallel, and that the distances between corresponding points on each curve closely resemble the differences in the proportions of the homologous antibodies. The interpretation of these experiments is that the inhibition observed with immunoglobulin mixtures is almost exclusively due to the antibody fraction possessing the idiotype. The nonspecific IgG in the mixture does not significantly influence the inhibition, even when present in a 1,000-fold excess. It was concluded from these experiments that the inhibition of idiotypic binding provides a direct estimate of the proportion of molecules possessing the idiotype within an immunoglobulin mixture. A proportion of 0.1% appeared to be the limit of detectability.

The proportion of molecules with A5A idiotype was determined in the antisera of 119 mice immunized with Group A streptococci. High proportions were seen only in mice bearing A/J genome, whereas low proportions were observed in several other strains. Illustrated in Fig. 4 are the inhibition curves obtained with antisera representative for strains A/J, BALB/cJ, and C57L/J. The antisera were diluted to the appropriate antibody concentrations and the inhibi-
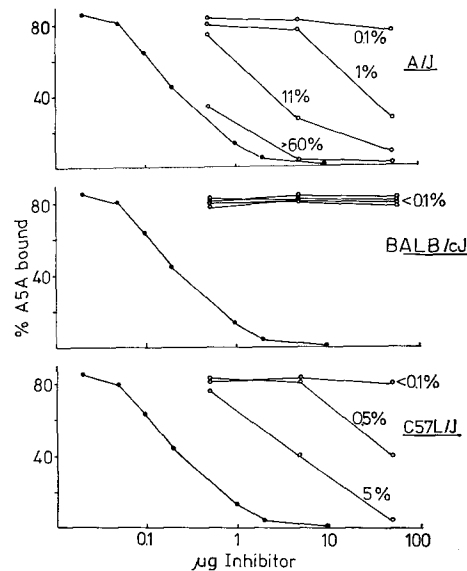

FIG. 4. Inhibition of the idiotypic binding between 20 μl of anti-A5A, 1:250 dilution, and 20 ng of [^{125}I]antibody A5A. The reference inhibition curve of cold antibody A5A (●—●) is included in each frame. Inhibitors were antisera to A-CHO from strain A/J (upper frame), from strain BALB/cJ (middle frame), and from strain C57L/J (lower frame). These antisera have been diluted to contain 0.5, 5, and 50 μg of antibody to A-CHO per test tube, respectively. The proportions given with each inhibition curve designate the proportions of antibody molecules with A5A idiotype, calculated from each inhibition curve.

tion for each antiserum was determined with amounts containing 0.5, 5, and 50 μg of anti-CHO antibody, respectively. From each inhibition curve the proportion of molecules with A5A idiotype was calculated by comparison with the reference inhibition curve of cold antibody A5A. Antibodies from strain A/J show a wide range of inhibitory capacities, indicating that the proportions of molecules with A5A idiotype vary from less than 0.1 % to more than 60 % of the total anti-CHO antibody in individual A/J mice. No inhibition was seen with the antibodies from strain BALB/cJ, indicating that the proportion of molecules with A5A idiotype is less than 0.1 % in each mouse. Antibodies from strain C57L/J are included here as an example for strong interstrain cross-specificity. Proportions of up to 5 % were observed.

The histogram in Fig. 5 summarizes the results of inhibition experiments performed with the antibodies of eight inbred strains excluding strain A/J. The abscissa gives the proportions of molecules with A5A idiotype in percent of the total antibody. Each box represents a single mouse. In strains BALB/cJ, CE/J, SWR/J, and C57BL/6J the proportion was <0.1 % for each mouse tested. Mice of strains SJL/J and DBA/1J occasionally produce a higher proportion of antibodies with A5A idiotype, indicating weak interstrain cross-specificity. Strong interstrain cross-specificity was observed for strains DBA/2J and C57L/J. Most mice of these strains produced significant proportions of antibodies with A5A idiotype. However, the proportions observed in strains other than A/J never exceeded 5 % of the total antibody.

Among the four strains that showed no interstrain cross-specificity, strain BALB/cJ was chosen for the production of F_1 hybrid mice with strain A/J. The reason for choosing strain BALB/cJ was that the magnitude of its immune response to A-CHO nearly equaled that of strain A/J (5), so that (A/J ×
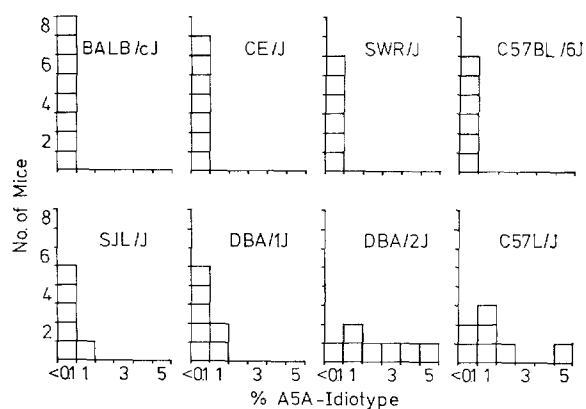
FIG. 5. Histogram of the expression of the A5A idiotype in eight inbred strains of mice. Each box represents the result on the antiserum to A-CHO from a single mouse. The abscissa gives the proportion of antibody molecules with A5A idiotype, as determined by inhibition of idiotypic binding (see Fig. 4).

BALB/cJ)F_1 hybrids could be expected to produce similar amounts of A/J- and BALB/cJ-derived antibodies. The histogram in Fig. 6 summarizes the results of inhibition experiments performed with the antisera to A-CHO of 19 BALB/cJ mice, 28 A/J mice, and 18 (A/J × BALB/cJ)F_1 hybrid mice. The abscissa gives the proportions of antibody molecules with A5A idiotype in percent of the total antibody. This proportion was less than 0.1 % in the antibodies of all of 19 BALB/cJ mice, whereas 27 out of 28 A/J mice and 15 out of 18 (A/J × BALB/cJ)F_1 hybrids produced a significant proportion of molecules with this idiotype. This is a clear indication that the A5A idiotype is the phenotypic expression of genes present in the A/J genome but absent from the BALB/cJ genome.

It can also be seen from Fig. 6 that the quantitative expression of antibodies with A5A idiotype in mice bearing A/J genome is subject to great individual variation. It should be mentioned that the antisera used for the inhibition experiments were taken after the second immunization series of each mouse (5). Therefore, they represent a momentary state within the course of the immune response. However, the distribution of the proportions appears to be nonrandom as indicated by the three peaks in the low, intermediate, and high ranges, respectively.

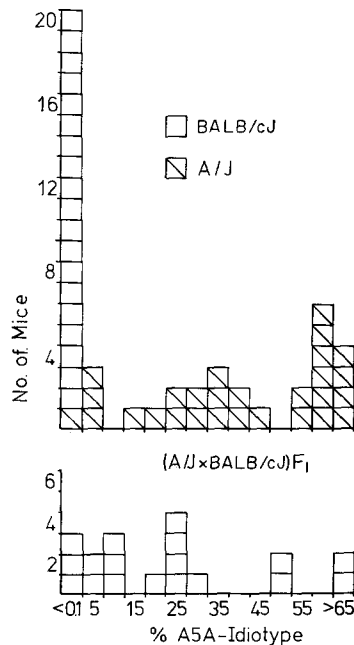
Fig. 6. Histogram of the expression of the A5A idiotype in A/J mice, BALB/cJ mice, and (A/J × BALB/cJ)F_1 hybrid mice. Each box represents the result on the antiserum to A-CHO from a single mouse. The abscissa gives the proportion of antibody molecules with A5A idiotype, as determined by inhibition of idiotypic binding (see Fig. 4).

Experiments identical with that performed to determine the expression of the A5A idiotype were done on the A2C idiotype. The proportions of antibody molecules with A2C idiotype were determined in the sera of 117 mice immunized with Group C streptococci. The results of these inhibition experiments were quite different from that obtained with the A5A idiotype and are summarized in Table I. In the antibodies to C-CHO from 69 mice belonging to 8 inbred strains excluding strain A/J, the proportions of molecules with A2C idiotype were less than 0.1%, indicating the complete lack of interstrain cross-specificity. Furthermore, only 3 out of 27 A/J mice and 4 out of 21 (A/J × BALB/cJ)F₁ hybrids produced significant proportions of antibody molecules with A2C idiotype. However, in those rare mice that expressed the A2C idiotype the proportions were not significantly lower than that of the

TABLE I

Frequency and Proportions of the A2C Idiotype in Mice Immunized with Group C Streptococci

Mice	no. tested	no. < 0.1%	no. > 0.1%	(A2C idiotype)
				%
A/J	27	24	3	(21, 25, >65)
(A/J × BALB/cJ)F₁	21	17	4	(5, 26, 27, 41)
BALB/cJ	20	20	–	
CE/J	6	6	–	
SWR/J	9	9	–	
C57BL/6J	7	7	–	
SJL/L	7	7	–	
DBA/1J	6	6	–	
DBA/2J	7	7	–	
C57L/J	7	7	–	

A5A idiotype. Thus, the major difference between idiotypes A5A and A2C was in their frequency of expression. This difference in the frequency of expression between the A5A and A2C idiotypes suggests that each clone may possess its own special frequency of expression in an inbred strain of mice.

Idiotype-Allotype Linkage in Heterozygous Mice.—Studies on the association of the allotypes in the V region and the allotypes in the C region of the rabbit heavy chain have provided evidence for the genetic linkage between the V and C gene loci coding for antibody polypeptide chains (23, 24). These observations have been supported by the detection of recombinations between rabbit V and C region allotypes (25, 26).

In the mouse, no serologic V region marker comparable to that of the rabbit heavy chain is known. The present studies, however, suggest that idiotypes are inherited in a strain-specific fashion and thus behave as allelic markers for the V regions of mouse antibody polypeptide chains. Therefore, a test system was developed to investigate the association of idiotypes and allotypes in the antibodies of heterozygous mice. As was shown above, antibodies to A-CHO

from $(A/J \times BALB/cJ)F_1$ hybrids usually contain a population of molecules possessing the idiotype of the A/J clone A5A. The determination of the heavy chain constant region allotype of this population would provide information on the genetic linkage between V and C genes coding for the A5A heavy chain. These allotypes are determined by selectively adsorbing from the F_1 antisera the antibody population with A5A idiotype to solidified anti-A5A antiserum. Subsequently the nonbound antibodies and serum proteins are removed by washing, and the washed complexes are exposed to specifically purified and radiolabeled antibodies against A/J (anti-A) and BALB/cJ (anti-BALB) heavy chain C region allotypes, and against mouse fragment Fc (anti-Fc) for control. The binding of each of these antibodies to the antibody population with A5A idiotype was then determined. Similar experiments were performed with the A/J and $(A/J \times BALB/cJ)F_1$ hybrid mice that expressed the A2C idiotype.

In order to eliminate allotypic cross-reactions between A/J and BALB/cJ IgG, the purified antiallotype antibodies were adsorbed to glutaraldehyde cross-linked sera of the heterologous strains. This adsorption removed 55% of the antibodies to BALB/cJ allotypes (anti-BALB). No adsorption occurred with the antibodies to A/J allotypes (anti-A) because they were derived from BALB/c mice.

In preliminary experiments the isolated antiallotype and anti-Fc antibodies were tested for their capacity to bind to Group A antibodies which were adsorbed to Group A streptococci. This adsorption does not select for an antibody population with a special idiotype as does adsorption to solidified anti-idiotype antisera. As shown at the top of Table II, antibody A5A binds maximally 42% of anti-A and 65% of anti-Fc, whereas pooled A/J antibodies bind maximally 68% of anti-A and 78% of anti-Fc. This indicates that anti-A (of unknown class specificity) detects allotypic determinations on more than one heavy chain class, only one of them being represented by antibody A5A. The specificity of the reagents is brought out by the binding of each antiallotype antibody to anti-CHO antibodies from the homologous strain and the lack of binding (<2%) to anti-CHO antibodies from the heterologous strain.

Summarized in Table II are the idiotype-allotype association data on the 15 $(A/J \times BALB/cJ)F_1$ hybrids that expressed the A5A idiotype. Included for comparison are the results of six A/J mice. It is evident that in each heterozygous mouse the antibody population was A5A idiotype exclusively binds antibodies to A/J allotypes. No binding of antibodies against BALB/cJ allotypes was observed in any of the 15 heterozygous mice. This is a clear indication for the linked genetic transmission of the V genes coding for the idiotype and the C genes coding for the heavy chain allotype of antibody clone A5A. It is noteworthy that in most of the mice the proportions of anti-A and anti-Fc bound to 10 ng of antibody with A5A idiotype are somewhat greater than that bound to 10 ng of antibody A5A. Although this could be explained by inaccuracy of the method, the tendency to higher proportions

suggests some nonspecific binding in this assay. Another possible explanation is the occurrence of the A5A idiotype with antibody molecules that belong to heavy chain classes different from that of antibody A5A (8).

Summarized in Table III are the idiotype-allotype association data on the

TABLE II

Binding of Purified Antiallotype and Anti-Fc Antibodies to Group A Antibodies with A5A Idiotype in the Sera of A/J Mice and (A/J × BALB/cJ)F_1 Hybrids

Antibodies	% of [125I]antibodies bound*		
	Anti-A	Anti-BALB	Anti-Fc
Adsorbed to Group A strep:‡			
A5A	42	1	65
Pooled A/J	68	1	78
Pooled BALB/cJ	2	59	75
Pooled IgG	1	2	1
Adsorbed to solidified anti-A5A:§			
A5A	41	0	65
Pooled A/J	48	1	74
Pooled BALB/cJ	1	0	1
Pooled IgG	0	1	1
A/J mouse 1	40	1	72
2	49	1	74
3	50	0	78
4	42	1	71
5	51	0	79
6	54	2	78
(A/J × BALB/cJ)F_1			
mouse 1	49	1	68
2	48	1	69
3	50	2	74
4	42	1	71
5	48	0	74
6	51	2	70
7	50	2	78
8	49	1	75
9	47	0	70
10	45	0	74
11	41	2	70
12	41	1	69
13	42	2	69
14	49	1	72
15	41	0	72

* 50 ng of radiolabeled antiallotype antibodies and 100 ng of anti-Fc antibody used in each test. These amounts are slightly less than equivalent for 10 ng of anti-CHO antibody, so that the percent values represent maximal binding.

‡ 10 ng of antibody adsorbed to Group A streptococci used in each test.

§ 10 ng of antibody adsorbed to solidified anti-A5A used in each test.

TABLE III

Binding of Purified Antiallotype and Anti-Fc Antibodies to Group C Antibodies with A2C Idiotype in the Sera of A/J Mice and (A/J × BALB/cJ)F₁ Hybrids

Antibodies	% of [¹²⁵I]antibodies bound*		
	Anti-A	Anti-BALB	Anti-Fc
Adsorbed to Group C strep:‡			
A2C	53	2	69
Pooled A/J	65	1	75
Pooled BALB/cJ	1	61	75
Pooled IgG	1	1	0
Adsorbed to solidified anti-A2C:§			
A2C	45	0	68
Pooled IgG	2	1	1
A/J mouse 1	49	1	74
2	51	0	72
3	49	2	75
(A/J × BALB/cJ)F₁			
mouse 1	48	2	72
2	49	1	70
3	48	1	69
4	51	2	75

* 50 ng of radiolabeled antiallotype antibodies and 100 ng of anti-Fc antibody used in each test. These amounts are slightly less than equivalent for 10 ng of anti-CHO antibody, so that the percent values represent maximal binding.

‡ 10 ng of antibody adsorbed to Group C streptococci used in each test.

§ 10 ng of antibody adsorbed to solidified anti-A2C used in each test.

three A/J mice and the four (A/J × BALB/cJ)F₁ hybrids that expressed the A2C idiotype. The results were similar to that obtained with the A5A idiotype. None of the antibodies with A2C idiotypic specificity bound a significant amount of anti-BALB. The proportions bound of anti-A and anti-Fc were generally somewhat greater than that bound by antibody A2C.

Taken together with the idiotype-allotype association data on the A5A idiotype, the experiments suggest that the expression of both clones investigated here is linked to the A/J heavy chain C region locus.

DISCUSSION

Together with a number of previous reports the experiments presented here indicate that idiotypes are useful to study antibody V gene expression and genetics (4, 5, 7–9). It appears now fairly well documented that idiotypes are inherited V region markers, but this inheritance has thus far been demonstrated for certain antibodies only. Such antibodies were of limited heterogeneity (4), or raised in genetically similar animals (7), or both (5, 8, 9). There seems to be another unknown requirement, because antibodies to the capsular

polysaccharides of pneumococci have failed to demonstrate inherited idiotypes, although they were raised in colony-bred rabbits and are commonly restricted in their heterogeneity (27).

The following discussion is based on the assumption that the idiotypic antisera which were developed against monoclonal mouse antibodies identify the very same clonal product in the antisera of other mice. This question obviously cannot be answered before amino acid sequences of antibodies with identical idiotypes have been compared. However, the experiments presented in Figs. 2 and 3 suggest that the technique of inhibition of idiotypic binding detects identical idiotypes only. In contrast, a systematic analysis of various procedures for the study of idiotypic cross-reactions has shown that direct idiotypic binding is less specific.[2] Without the presence of the homologous antibody, idiotypic antisera bind antibodies of definitely different idiotypes, presumably through partially shared determinants. Direct idiotypic binding was employed for the idiotype-allotype association test in this report and for inhibition of plaque formation in another report (8). These results have to be interpreted with the proper precautions. The choice of the animal for the production of anti-idiotypic antisera appears to be of less importance. The comparison of the results obtained with heterologous (guinea pig anti-mouse) anti-idiotypic antisera to those previously obtained with isologous (A/J anti-A/J) anti-idiotypic antisera revealed no qualitative differences (5).

The observations on the idiotypes of two mouse antibody clones revealed common as well as distinct features in their patterns of expression and inheritance. Common to both antibody clones investigated here was their strain specificity and the observation that their idiotypes remained associated with their heavy chain C region allotype in heterozygous mice. Common to both clones was also the quantitative variation in their expression in individual mice. Both clones differed, however, markedly in their frequency of expression in the investigated mouse population. Clone A5A was expressed in more than 80% of mice bearing A/J genome, clone A2C in less than 20%.

The quantitative expression of both clones in the antibodies of individual mice revealed great variability, which appeared to be nonrandom as suggested by the three peak distribution observed with clone A5A. The significance of this distribution is not at all clear, but the variability among genetically similar animals suggests that factors not associated with the germ line of the strain control the quantitative expression of antibody clones. This individual variability cannot, however, account for the observed strain specificity of clonal idiotypes. The association of both idiotypes with the A/J genome was statistically highly significant with a P value of < 0.0005 for A5A and a P value of < 0.005 for A2C (χ^2 test used).

In a previous report (5) the question was raised whether the strain specificity of idiotypes was caused by genetic polymorphism of antibody V genes, or by

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[2] Eichmann, K. Manuscript in preparation.

suppression of certain clones in certain strains, possibly through their cross-reactivity with histocompatibility antigens (28). The latter seems ruled out by the observation that both the A5A and the A2C idiotypes are expressed in (A/J × BALB/cJ)F$_1$ hybrids. If the lack of expression of both clones in strain BALB/cJ were caused by histocompatibility antigens, the same suppression should have occurred in (A/J × BALB/cJ)F$_1$ hybrids. Therefore, without the assumption of other hypothetical regulary systems, V gene polymorphism appears to be the only alternative. This polymorphism might be general because rabbit heavy chain V genes are known to be polymorphic for some time (29, 30), and recent evidence suggests polymorphism of mouse κ-chain V genes (31). On the other hand, amino acid sequence studies have clearly demonstrated the existence of nonallelic V genes specifying the V region subgroups (32–34). Therefore, on the basis of the present evidence, one has to assume that the genes coding for V region subgroups exist in two or more allelic forms.

In the rabbit, the genes specifying the heavy chain V and C regions are known to be closely linked (23–26) and a general arrangement of V and C gene loci in closely linked but separate clusters has been suggested (35). The present experiments are in agreement with these previous observations, in demonstrating a similar linkage of the V genes coding for the idiotype and the C genes coding for the heavy chain C region allotype of two mouse antibody clones. In the idiotype-allotype association experiments, germ line recombinations would have been detected by the reassociation between idiotype and allotype for all the clonal antibody molecules, whereas somatic recombination would have been detected by such reassociation in a fraction of the antibody molecules. However, no recombinant molecules were found. As was pointed out before, direct idiotypic binding as employed in the idiotype-allotype association assay detects antibody molecules with similar but non-identical idiotype. Therefore, only those mice were tested that were previously shown by inhibition of idiotypic binding to possess antibody molecules with the investigated idiotypes. Nevertheless, some background of recombinant molecules was expected, because (A/J × BALB/cJ)F$_1$ hybrids should possess antibody molecules composed of the A5A light chain and a BALB/c-derived heavy chain. Such molecules would have been bound to the solidified anti-idiotype antiserum by their light chain determinants, and would mimic recombinant molecules by their heavy chain allotype. The complete lack of all recombinant molecules was taken as an indication that the light chain idiotype did not play a major role in the idiotype-allotype association experiments. Therefore, it can be concluded that the results are primarily indicative for the linkage of V and C genes coding for the clonal heavy chains.

The most puzzling result of this investigation was the difference in the frequency of expression between the two antibody clones in the same inbred strain. Although obviously a number of alternative explanations for this

observation can be thought of, it appears most attractive to speculate on possibilities that are provided by the translocation hypothesis (35). The apparent infrequency of somatic recombinations between V and C genes in rabbits (22, 36) and in mice strongly indicates that translocation of V gene episomes between homologous chromosomes is an infrequent event. Thus it appears that translocation is rare or impossible over large distances. Correspondingly, short distances may facilitate translocation, and the possibility exists that the frequency of translocation is a function of the distance between the original position of the V gene and its final position next to the C gene to which translocation has to take place. If this is the case, the frequency of expression for a clone is regulated by the distance between the V and C genes that have to cooperate in coding for its polypeptide chains. Such an assumption has the advantage that besides antigen stimulation and statistical events on the DNA level no secondary regulatory mechanisms are required. Aside from the observations on clones A5A and A2C, this extension of the translocation hypothesis readily provides an explanation for the gradient in the expression of heavy chain classes in normal immunoglobulin and myeloma proteins (37). A similar gradient should exist for the expression of V genes, and was in fact observed for the rabbit κ-chain V region subgroups (38, 39). Furthermore, antibody clones of guinea pigs immunized with dinitrophenyl-lysine showed a gradient in their frequency of expression.[3]

Because of the preferential selection and neglect of V region subgroups by antigen (38, 39), the mere determination of the frequency of expression of idiotypes cannot itself provide information on the positions of V genes relative to the C gene cluster. However, the above considerations suggest that V gene loci may in fact differ in their distances from the C gene cluster, and should therefore segregate upon recombination events. Two recombinations between V and C genes have been described in rabbits (25, 26), and it should be feasible to identify a larger number of recombinants in mice. This may provide an approach to the mapping of antibody V gene loci.

SUMMARY

The inheritance of idiotypes was investigated using idiotypic antisera against two monoclonal antibodies to streptococcal carbohydrates derived from A/J mice. Each of the two idiotypes was characterized by a special frequency of expression. One of the idiotypes was expressed in more than 80% of A/J mice, the other in less than 20%.

The idiotypes of both antibodies were strain specific and were transmitted to (A/J × BALB/cJ)F_1 hybrid mice. Furthermore, both idiotypes remained associated with the A/J heavy chain C region allotype in (A/J × BALB/cJ)F_1 hybrid mice. The results suggest that idiotypes are specified by allelic V genes,

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[3] Williamson, A. R. Personal communication.

and that the heavy chain idiotype locus is linked to the heavy chain allotype locus.

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