ANTI-IMMUNOGLOBULIN ANTIBODIES

I. Expression of Cross-Reactive Idiotypes and *Ir* Gene Control of the Response to IgG_{2a} of the b Allotype

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It has become increasingly clear that immunoglobulin molecules expressed on precursors of antibody-secreting cells act not only as membrane receptors for antigen but also as sites for the regulatory action of specific antibodies (1, 2) and T lymphocytes (3–6). One especially striking example of this Ig-specific regulatory control is provided by the chronic suppression of IgG_{2a} antibodies of the b allelic type (IgG_{2a}^{b})[1, 2] in (SJL × BALB/c)F_1 hybrids maternally or neonatally treated with anti-allotype antibodies (7). Because the regulation of IgG_{2a}^{b} may provide a particularly powerful system to study the normal control of Ig class and allotype expression, it is of considerable importance to delineate the genetic regulation and idiotypic character of anti-allotype antibodies in general, and of anti-IgG_{2a}^{b} antibodies in particular. Lieberman and Humphrey (8, 9) have previously demonstrated that antibody responses to the a allelic forms of IgA (IgA^{a}) and IgG_{2a} (IgG_{2a}^{a}) are regulated by major histocompatibility complex (MHC) immune response (*Ir*) genes. The responses to IgA^{a} determinants depend on a gene (or genes) in the *I-A* subregion of the MHC with mice of *a*, *k*, *p*, *r*, and *s* haplotypes being responders. Responses to IgG_{2a}^{a} determinants are regulated by an *I-B*-encoded *Ir* gene with responder alleles being found in mice of *b*, *p*, *r*, *s*, and *v* haplotypes.

In this communication, we report data that indicate that responses to the b allelic form of IgG_{2a} are also regulated by MHC-encoded *Ir* genes. Furthermore, we find that all responder strains produce anti-IgG_{2a}^{b} antibodies that share common idiotypes (cross-reactive idiotypes [IdX]), no matter what allelic forms of the Ig heavy (H)-chain-constant region (*Igh-C*) genes they possess. It appears that the variable regions of these anti-allotype antibodies are encoded by a family of highly conserved genes.

Materials and Methods

Animals. 8-wk-old mice were used in these experiments. Their *MHC* and *Igh-C* types are presented in Table I.

* Recipient of fellowship 1F32 AI 05568-02 from the Public Health Service, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

[1] IgG_{2a} of the b allotype is designated Ig-1B in the Herzenberg nomenclature. Similarly, IgG_{2a}^{a} is Ig-1a, IgA^{a} is Ig-2a, IgG_{2b}^{a} is Ig-3a, and IgG_{1}^{a} is Ig-4a.

[2] *Abbreviations used in this paper:* H, heavy; HA, hemagglutination; HI, inhibition of hemagglutination; IdX, cross-reactive idiotype(s); IgA^{a}, the a allelic type of IgA; IgG_{2a}^{a}, the a allelic type of IgG_{2a}; IgG_{2a}^{b}, the b allelic form of IgG_{2a}; Igh-C, Ig H-chain-constant region; Igl-V, Ig light-chain-variable region; Igh-V, Ig H-chain-variable region; KLH, keyhole limpet hemocyanin; L, light; MHC, major histocompatibility complex; Ova, ovalbumin; RIA, radioimmunoassay; SRBC, sheep red blood cells.

Myeloma Proteins. CBPC101 (IgG_{2a}^b) myeloma protein was obtained from ascitic fluid of C.B20 mice that bore the CBPC101 myeloma tumor. It was purified on a protein A-Sepharose 4B column (Pharmacia Fine Chemical Co., Div. of Pharmacia, Inc., Piscataway, N. J.). UPC10 and LPC1 (IgG_{2a}^a), MOPC195 (IgG_{2b}^a), MOPC31C (IgG_1^a), and UPC61 (IgA^a) myeloma proteins were obtained from Dr. M. Potter (National Cancer Institute, National Institutes of Health, Bethesda, Md.).

Products of hybridomas No. 10-3.6, Ig(1b)5.7.1, Ig(1b)3.1, Ig(1b)2.9, and Ig(1b)4.7 were obtained from Dr. V. Oi and Dr. L. Herzenberg (Stanford University, Stanford, Calif.) and S1G.1E and S1A.1A from Dr. M. Bosma (Institute for Cancer Research, Fox Chase, Pa.).

Immunization of Mice.

PREPARATION OF ANTI-IgG_{2a}^b ALLOTYPE ANTIBODY IN VARIOUS STRAINS OF MICE. Anti-allotype antibodies were prepared by immunization of mice with 75 μg of CBPC101 myeloma protein emulsified in complete Freund's adjuvant (CFA). This was followed 5 d later by a similar dose of myeloma protein in incomplete Freund's adjuvant and then by one to six weekly injections of 75 μg of myeloma protein in saline.

PREPARATION OF ANTI-IDIOTYPE (Id) SERUM. BALB/c anti-IgG_{2a}^b allotype antibodies from an individual mouse and pooled C.AL20, P/J, and DBA/2 anti-IgG_{2a}^b allotype antibodies were purified by affinity chromatography on a column of CBPC101-Sepharose 4B according to a previously described technique (10). These purified anti-allotype antibodies were coupled to keyhole limpet hemocyanin (KLH) at a 1:1 ratio as previously described (10). BAB.14, CXBI, and B.C8 mice were immunized with the anti-allotype-KLH conjugate according to the same schedule as that used to raise anti-allotype antibodies. These antisera were made specific for Id by adsorption with BALB/c, BALB.B, P/J, C.AL20, or DBA/2 immunoglobulins as described by Lieberman and Humphrey (8).

Coating of Sheep Erythrocytes (SRBC). SRBC were coated with myeloma proteins, with purified BALB/c, C.AL20, P/J, and DBA/2 anti-CBPC101 antibodies and with C57BL/6 Ig by the chromic chloride method using a concentration of 1 mg/ml of the appropriate protein (6).

Determination of Hemagglutinin (HA) Titers. HA titers of antibodies specific for IgG_{2a}^b allotypic determinants were measured in microtiter plates using SRBC that were coated with CBPC101 or C57BL/6 Ig. The titer of anti-class or anti-allotype antibodies specific for Igh-C determinants of the a allotypic form was determined using UPC10, MOPC195, MOPC31C, and UPC61-coated SRBC. In each case, the titer recorded is $1/\log_2$ of the highest dilution of antisera that caused agglutination.

Determination of Serum IdX Titer. A hemagglutination-inhibition (HI) method described previously (2) was used to test sera for Id. In these experiments, specifically purified BALB/c anti-IgG_{2a}^b antibodies were coupled to SRBC. The capacity of various sera to inhibit agglutination of these cells by BAB.14, CXBI, or B.C8 anti-(BALB/c anti-IgG_{2a}^b) antibodies was determined. The titer reported is $1/\log_2$ of the highest dilution that caused inhibition of hemagglutination.

Radioimmunoassay. Purified BALB/c, C.AL20, P/J, and DBA/2 anti-IgG_{2a}^b antibodies were tritiated according to a technique described by Wilder et al. (11). The specific activity was ~0.05 μCi/μg protein.

The ability of ^{3}H-BALB/c, -C.AL20, -P/J, and -DBA/2 anti-IgG_{2a}^b antibodies to be bound by BAB.14, CXBI, or B.C8 anti-Id antisera was determined as follows: Microtiter plates were coated with various dilutions of anti-Id antisera by overnight incubation followed by three washings with saline. The plates were then incubated with 50% fetal calf serum (FCS) in saline. After three washings with saline, they were incubated with ^{3}H-anti-IgG_{2a}^b for 3 h. The plates were then exhaustively washed and dried. Radioactivity on plates was measured in a liquid scintillation spectrometer (Beckman Instruments, Inc., Fullerton, Calif.). To determine the capacity of various anti-IgG_{2a}^b antisera to inhibit the binding of ^{3}H-anti-IgG_{2a}^b by anti-Id antibodies, plates that were already coated with BAB.14, CXBI, or B.C8 antisera were incubated for at least 3 h with various dilutions of the inhibitory sera prior to the addition of ^{3}H-antibodies.

Isoelectric Focusing. Anti-IgG_{2a}^b antisera were analyzed by isoelectric focusing according to a previously described technique (12). Gels were overlaid with a ^{125}I-labeled Fc fragment of

CBPC101 myeloma protein, kindly donated by Dr. M. Stanislavsky, Centre National de Recherche Scientifique, Villejuif, France.

Results

Genetic Control of Production of Anti-Allotype (IgG_{2a}^b) Antibody. Various strains of mice were injected with CBPC101 myeloma protein, and the titer of anti-allotype antibody was determined by HA assay. In those cases in which sera agglutinated CBPC101- and C57BL/6-Ig-coated SRBC at a similar dilution, we concluded that anti-allotype antibody was present, whereas for those cases in which the sera agglutinated CBPC101-coated SRBC but not C57BL/6-Ig-coated SRBC, we concluded that antibodies specific for the Id of the CBPC101 myeloma protein (anti-Id CBPC101) were present.

Mice of the *d* (i.e., BALB/c, DBA/2, and C.AL20), *r* (i.e., RIII), *p* (i.e., P/J), *q* (i.e., DBA/1J), and *s* (i.e., SJA) haplotypes made very strong anti-allotype antibody responses as a result of immunization with CBPC101. Among mice of the *b* type, BALB.B., B.C8, CXBJ, and CXBG made strong responses; however, only one of five 129/SV mice made anti-allotype antibody, whereas two mice made only anti-Id CBPC101 antibody. Mice of the *k* (i.e., CBA/J, CBA/N, BALB.K, C3H/HeN, CE/J, C58/J, and AKR), *m* (i.e., AKR.M), *a* (i.e., A/He), a^l (i.e., AL/N), *u* (i.e., PL/J), and *z* (i.e., NZW/J) haplotypes made no, or only a very poor, response (Table I). It should be noted that the HA titer of CBA/N serum probably does not represent anti-allotype antibody because this serum agglutinates UPC-10 (IgG_{2a}^a)-coated SRBC to the same extent as it agglutinates CBPC101-coated SRBC.

The importance of MHC genes in this response is shown by the fact that an excellent correlation between MHC type and anti-allotype response exists. Furthermore, among a series of congenic strains, including BALB/c ($H\text{-}2^d$), BALB.B ($H\text{-}2^b$), and BALB.K ($H\text{-}2^k$), the $H\text{-}2^d$ and $H\text{-}2^b$ strains respond, although the $H\text{-}2^k$ strain does not. Although these results indicate that the critical genes are within the MHC, they do not map such genes more precisely. We have been unable, thus far, to carry out intra-MHC mapping largely because strains which themselves possess IgG_{2a}^b (e.g., C57BL/10) do not make anti-allotype antibody to CBPC101; thus, the B10 recombinant series cannot be employed for mapping.

Characterization of Anti-Id Antibodies Against BALB/c Anti-Allotype (IgG_{2a}^b) Antibodies. BAB.14 and CXBI mice were immunized with affinity chromatography-purified anti-CBPC101 antibodies coupled to KLH. The anti-CBPC101 antibodies used for immunization originated from a single BALB/c mouse. Four BAB.14 and three CXBI mice developed high-titered antibodies which agglutinated SRBC to which BALB/c anti-CBPC101 antibodies had been coupled. These sera were pooled and found to have little or no activity against SRBC coupled with UPC10 (IgG_{2a}^a), MOPC195 (IgG_{2b}^a), MOPC31C (IgG_1^a), and UPC61 (IgA^a). The agglutination titer of the pool of BAB.14 antisera for SRBC coupled with BALB/C anti-CBPC101 antibody was 2.6×10^5, whereas that of CXBI pooled serum was 1.3×10^5 (Table II). Furthermore, BAB.14 and CXBI normal sera, BAB.14 anti-(BALB/c anti-ovalbumin [Ova]) serum, CBPC101 (10 mg/ml), and C57BL/6 Ig (2 mg/ml) caused little or no agglutination of SRBC that were coated with purified BALB/c anti-CBPC101 antibody. Thus, the ability of anti-(BALB/c anti-CBPC101) antibody to cause agglutination of anti-CBPC101-coated erythrocytes cannot be explained by the

TABLE I

Genetic Control of Production of Anti-Allotype (IgG_{2a}^b) Antibodies

Strain*	MHC type	Igh-C type	HA titer (log_2) on SRBC coated with		
			CBPC101	C57BL/6 Ig	UPC10 (IgG_{2a}^a)
BALB/c AnN	d	a	>12	>12	0
C.AL20	d	o	11	>12	1.5
DBA/2J	d	c	>12	>12	5.5
BALB.B	b	a	11	10	0
B.C8	b	a	11.5	8.5	0
CXBJ	b	a	>12	7	2
CXBG	b	a	10	>12	0
129/SV‡	b	a	>12	>12	0
BALB.K	k	a	0	0	0
C58/J	k	a	4	2	0
CBA/J	k	j	0	0	0
CBA/N	k	j	7	4	8
C3H/HeN	k	j	4	1.5	1
AKR/N	k	d	0	0	0
CE/J	k	f	2.3	1	2
A/He	a	e	1.5	0.5	2
AL/N	a^1	o	0	0	0
AKR.M	m	d	0	0	0
RIII	r	g	>12	>12	2
P/J	p	h	>12	>12	0
NZW/J	z	n	1.5	1	0
PL/J	u	j	3.1	5.4	2.8
DBA/1J	q	c	9	9	0
SJA	s	a	>12	>12	0

* Three to five mice were tested for each strain.

‡ Only one out of five mice made anti-allotype antibody.

binding of anti-CBPC101 to IgG_{2a}^b in the agglutinating sera. Rather, it must represent the activity of anti-Id antibody in the CXBI and BAB.14 anti-(BALB/c anti-CBPC101) antisera.

Anti-Id antibodies against anti-IgG_{2a}^b allotype antibodies were also obtained in B.C8 mice by immunization with BALB/c anti-CBPC101 antibodies. B.C8 mice possess the BALB/c(a) form of IgG_{2a}. Anti-Id antibodies were also obtained in B.C8 mice immunized with purified C.AL20, P/J, and DBA/2 anti-CBPC101 antibodies (Table III). The titer of anti-Id antibodies obtained in BAB.14 and CXBI mice was

TABLE II

Hemagglutination of SRBC Coated with BALB/c Anti-CBP101 Antibody By BAB.14 and CXBI Anti-Id Sera

Antiserum	SRBC coated with				
	BALB/c anti-CBPC101 antibody	UPC10	MOPC195	MOPC31	UPC61
CXBI anti-(BALB/c anti-CBPC101)	17*	3	3	2	3
CXBI normal serum	2	0	0	0	0
BAB.14 anti-(BALB/c anti-CBPC101)	18	2	4	3	1
BAB.14 anti-(BALB/c anti-Ova)	4	4	3	4	0
BAB.14 normal serum	1	0	0	0	0
CBPC101 (10 mg/ml)	3	0	0	0	0
C57BL/6 Ig (2 mg/ml)	0	0	0	0	0

* HA titer ($\log_2$).

TABLE III

HA Titer of Anti-Id Sera Raised in Various Strains of Mice Immunized with Purified Anti-CBPC101 Antibody

Immunization with	Strain in which anti-Id serum was raised	Anti-Id serum adsorbed with	
		Nothing	BALB/c IgG (3 mg/ml)
BALB/c anti-CBPC101 antibody*	BAB.14	18¶	17
	CXBI	17	15
	B.C8	7	7
		Nothing	C.AL20 (3 mg/ml)
C.AL20 anti-CBPC101 antibody‡	B.C8	6	5
		Nothing	P/J IgG (3 mg/ml)
P/J anti-CBPC101 antibody§	B.C8	5	3
		Nothing	DBA/c IgG (3 mg/ml)
DBA/2 anti-CBPC101 antibody‖	B.C8	5	5

* Indicator cells: SRBC coated with BALB/c anti-CBPC101 antibody.
‡ Indicator cells: SRBC coated with C.AL20 anti-CBPC101 antibody.
§ Indicator cells: SRBC coated with P/J anti-CBPC101 antibody.
‖ Indicator cells: SRBC coated with DBA/2 anti-CBPC101 antibody.
¶ HA titer ($\log_2$).

higher than the titer of anti-Id antibodies obtained in B.C8 mice. This may be related to the heightened immogenicity of a complex between IgG_{2a}^{b} and anti-CBPC101 antibody that can occur during immunization of BAB.14 and CXBI mice with BALB/c anti-CBPC101 antibody, but cannot occur in B.C8(IgG_{2a}^{a}) mice.

Utilizing a radioimmunoassay (RIA), excellent binding of ^{3}H-BALB/c, C.AL20, P/J, and DBA/2 anti-CBPC101-purified antibody by BAB.14 and B.C8 anti-Id sera was obtained (Fig. 1). The ^{3}H-BALB/c anti-CBPC101 antibody was also bound by

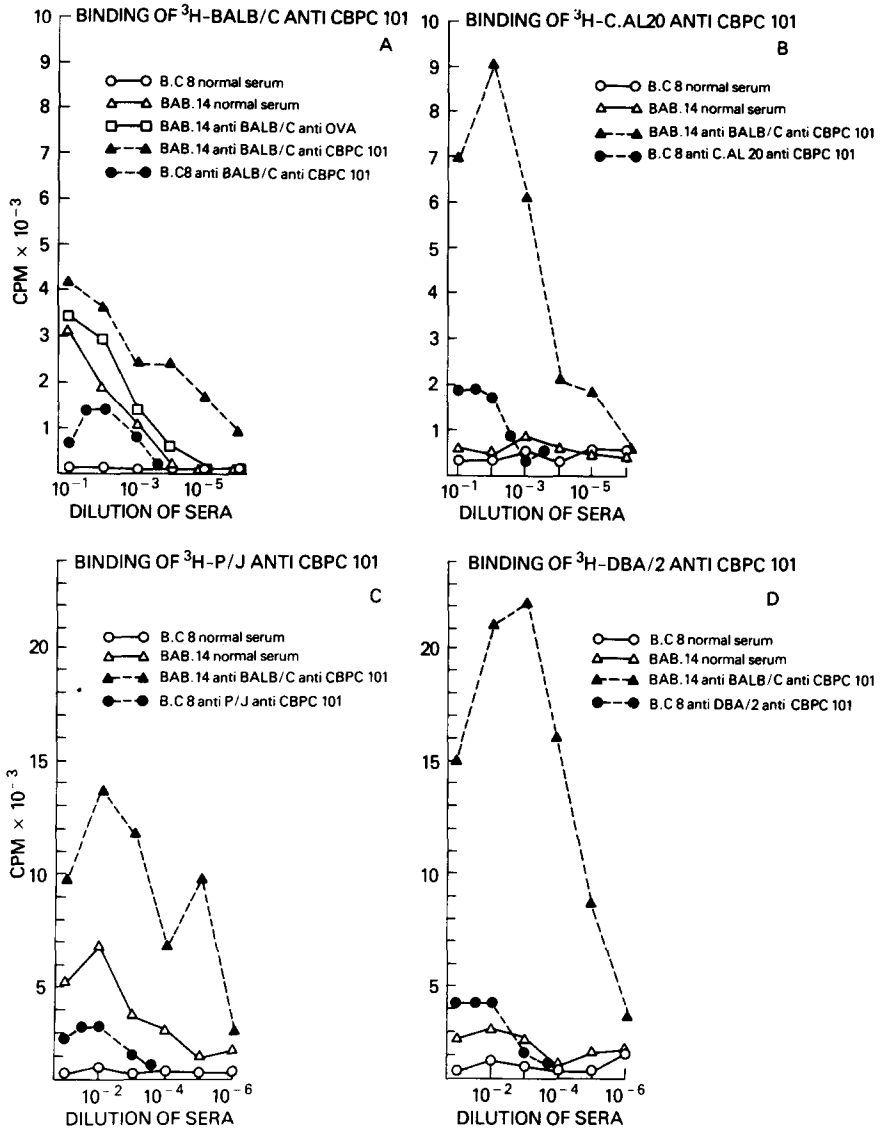

FIG. 1. Binding of ^{3}H-anti-CBPC101 antibodies by BAB.14 and B.C8 anti-Id antibodies. Microtiter plates were coated with varying dilutions of anti-Id antiserum or normal serum. The capacity of these sensitized plates to bind ^{3}H-anti-CBPC101 antibodies produced in BALB/c (A), C.AL20 (B), P/J (C), and DBA/2 (D) mice is illustrated.

normal BAB.14 serum and by BAB.14 antiserum specific for the Id of BALB/c anti-Ova antibody, as expected, because these sera contain $IgG_{2a}{}^{b}$ molecules. However, the binding of ^{3}H-BALB/c anti-allotype antibody to BAB.14 anti-(BALB/c anti-CBPC101) antibody was substantially greater than that of the control sera and could be observed at a 10^{-6} dilution; at such a dilution, the control sera had no binding activity. The ^{3}H-BALB/c anti-CBPC101 antibodies were significantly bound by B.C8 anti-Id serum but not by normal B.C8 serum (Fig. 1 A). The ^{3}H-C.AL20 and DBA/2 anti-CBPC101 antibodies were not significantly bound by BAB.14 and B.C8 normal sera but were strongly bound to BAB.14 and B.C8 anti-Id sera (Fig. 1 B and D). The ^{3}H-P/J anti-CBPC101 antibodies were significantly bound to BAB.14 normal serum diluted 10^{-2}. However, the binding of these labeled antibodies by BAB.14 and B.C8 anti-Id antisera was significantly higher than that of corresponding normal sera at all dilutions (Fig. 1 C).

These results indicate that immunization of BAB.14, CXBI, or B.C8 mice with purified anti-CBPC101 allotype antibody led to the production of antisera that contained antibodies against Id determinants of BALB/c, C.AL20, P/J, and DBA/2 anti-$IgG_{2a}{}^{b}$ allotype antibodies. Preincubation of ^{3}H-anti-CBPC101 antibody with unlabeled anti-CBPC101 or with the Fc fragment of CBPC101 strongly inhibited the subsequent binding of ^{3}H-antibody to both BAB.14 and B.C8 anti-Id antisera coated on microtiter plates (Table IV). This result indicates that the binding of Id by anti-Id is inhibited in the presence of antigen (IgG_{2a}) and thus suggests that the idiotypic determinants of the anti-allotype antibodies are associated with the antigen combining site.

Presence of IdX on Anti-($IgG_{2a}{}^{b}$)-Allotype Antibodies Produced by Various individual BALB/c Mice and by Mouse Strains of the b, d, p, q, r, and s MHC Haplotypes. Anti-allotype sera from nine individual BALB/c mice were used to inhibit subsequent

TABLE IV

Inhibition of Binding of ^{3}H-Anti-CBPC101 Antibody to B.C8 and BAB.14 Anti-Id Sera by Fc Fragments of CBPC101 Myeloma Protein

Inhibitors	Percent inhibition of binding in radioimmunoassay							
	Plates coated with B.C8 anti-Id serum				Plates coated with BAB.14 anti-(BALB/c anti-CBPC101)			
	^{3}H-BALB/c* anti-CBPC101 antibody	^{3}H-C.AL20‡ anti-CBPC101 antibody	^{3}H-P/J§ anti-CBPC101 antibody	^{3}H-DBA/2‖ anti-CBPC101 antibody	^{3}H-BALB/c anti-CBPC101 antibody	^{3}H-C.AL20 anti-CBPC101 antibody	^{3}H-P/J anti-CBPC101 antibody	^{3}H-DBA/2 anti-CBPC101 antibody
BALB/c anti-CBPC101 (1 mg/ml)	97	—	—	—	96	—	—	—
C.AL20 anti-CBPC101 (1.3 mg/ml)	—	85	—	—	—	95	—	—
P/J anti-CBPC101 (1 mg/ml)	—	—	89	—	—	—	93	—
DBA/2 anti-CBPC101 (0.4 mg/ml)	—	—	—	52	—	—	—	81
Fc fragment of CBPC101 (75 μg/ml)	98	95	97	96	98	88	95	94
UPC10 (10 mg/ml)	3	5	18	4	2	11	6	4

* Plates coated with B.C8 anti-(BALB/c anti-CBPC101).
‡ Plates coated with B.C8 anti-(C.AL20 anti-CBPC101).
§ Plates coated with B.C8 anti-(P/J anti-CBPC101).
‖ Plates coated with B.C8 anti-(DBA/2 anti-CBPC101).

binding of anti-Id antibody in HA and RIA tests to determine whether the Id expressed on the anti-allotype antibodies of the individual BALB/c mouse used to produce BAB.14 and CXBI anti-Id antibodies was also expressed on the anti-allotype antibodies of other BALB/c mice. As can be seen in Table V, all nine BALB/c mice produced a high titer of anti-allotype antibodies. Sera of each of these mice inhibited binding of CXBI anti-Id antisera in both HA and RIA tests. With BAB.14 anti-Id serum, six of the nine individuals showed a substantial inhibitory titer in RIA, although less than they had exhibited with the CXBI anti-Id antibody. These results suggest that idiotypic determinants were shared by anti-allotype antibodies produced in various individual BALB/c mice.

Anti-CBPC101 antisera from 12 responder strains of mice were tested for the presence of IdX by determining their ability to inhibit the reaction between B.C8 and BAB.14 anti-Id sera and SRBC that were coated with BALB/c anti-CBPC101 (Table VI). Anti-allotype antisera from each of these strains inhibited hemagglutination indicating the sharing of IdX by their anti-allotype antibodies. Similar results were obtained by RIA using ^{3}H-BALB/c anti-CBPC101 antibodies and BAB.14 anti-Id antiserm.

In general, mice of the *H-2*^{b} type developed lower IdX HI titers than did mice of other responder MHC types. The expression of IdX on anti-allotype antibody was observed in mice of each *Igh-C* type tested; this included the *a*, *c*, *g*, *h*, and *o* types. We could not determine the capacity of mice of the *b Igh-C* type to produce IdX because these mice possess IgG_{2a}^{b} and thus do not produce anti-allotype antibodies upon immunization with CBPC101.

The IdX was not expressed on a goat anti-IgG_{2a}^{b} anti-allotype antibody which was raised by immunization with the Fc fragment of CBPC101. Similarly, sera which were principally specific for idiotypic determinants of CBPC101 raised in responder

TABLE V

IdX on Anti-Allotype Antibodies from Several BALB/c Individuals

BALB/c mouse number	HA titer (log_2); SRBC coated with			HI titer (log_2)*		Inhibition of RIA‡	
	CBPC101	C57BL/6-Ig	UPC10	BAB.14 anti-Id serum	CXBI anti-Id serum	BAB.14 anti-Id serum	CXBI anti-Id serum
1	>12	>12	0	4	8	3	1,000
2	>12	>12	0	8	>8	100	1,000
3	>12	>12	0	8	>8	100	1,000
4	>12	>12	0	>8	>8	100	1,000
5	>12	>12	0	7	>8	30	1,000
6	>12	>12	0	3	>8	100	1,000
7	>12	>12	0	2	>8	<10	100
8	>12	>12	0	2	>8	<10	1,000
9	>12	>12	0	7	>8	30	1,000

* SRBC were coated with specifically purified BALB/c anti-CBPC101 antibodies.

‡ Plates were coated with a 1:50,00 dilution of either BAB.14 or CXBI anti-(BALB/c anti-CBPC101) and incubated with the following dilutions of anti-CBPC101 antisera from individual BALB/c mice: 1:3, 1:10, 1:30, 1:100, 1:300, and 1:1,000. The capacity of ^{3}H-BALB/c anti-CBPC101 antibodies to bind to the plate was then tested. Results are expressed as 1/highest dilution of serum causing >50% inhibition of binding.

TABLE VI

Expression of IdX on Anti-Allotypes Antibodies Produced in Various Strains of Mice

Strain Immunized	MHC type	Igh-C type	HI: SRBC coated with BALB/c anti-CBPC101; anti-Id produced in		Inhibition of binding of ^{3}H-BALB/c anti-CBPC101 by BAB.14 anti-(BALB/c anti-CBPC101)
			B.C8	BAB.14	
BALB/c	d	a	>8*	>8	1,000‡
BALB.B	b	a	3	6	10
B.C8	b	a	2	3	5
CXBG	b	a	4	6	30
CXBJ	b	a	3	4	3
129/SV	b	a	3	4	100
DBA/2	d	c	6	>8	1,000
DBA/1	q	c	>8	5	100
C.AL20	d	o	7	>8	1,000
RIII	r	g	6	>8	650
P/J	p	h	2	>8	650
SJA	s	a	>8	>8	1,000

* $1/\log_2$ of the highest dilution causing inhibition of hemagglutination.

‡ Microtiter plates were coated with a 1:50,000 dilution of BAB.14 anti-(BALB/c anti-CBPC101) antiserum and incubated with 1:3, 1:10, 1:30, 1:100, 1:300, 1:1,000, and 1:3,000 dilutions of anti-allotype serum from various strains. In some experiments, other dilutions of inhibitors were used. ^{3}H-BALB/c anti-CBPC101 was used for binding. The result reported is 1/highest dilution of anti-allotype serum causing >50% inhibition of binding.

strains (e.g., 129, CXBJ, BALB.B, and DBA/1) and in nonresponder strains lacked the IdX as tested by HA assay and by RIA.

Through the kindness of Dr. Vernon Oi, Dr. Leonard Herzenberg, and Dr. Melvin Bosma, we had the opportunity to study the expression of IdX on monoclonal anti-IgG_{2a}^b anti-allotype antibodies obtained from fusion of lymphocytes of BALB/c or SJA mice immunized with CBPC101. Each of these hybridoma products is specific for allotypic determinants of CBPC101. In four cases, more precise specificity assignments have been made. Both IG(1b)5.7.1 and Ig(1b)3.1 appear to identify C_{H3} domain determinants, whereas Ig(1b)2.9 has been assigned a C_{H2} domain specificity and Ig(1b)4.7, a hinge-region specificity (13). Each of the hybridoma antibodies, with the exception of Ig(1b)4.7, expressed a substantial HA titer for CBPC101 and C57BL/6-Ig-coated SRBC (Table VII). All of the HA-positive hybridoma antibodies inhibited the binding of BAB.14 anti-Id sera to anti-CBPC101 antibody, although the inhibitory titer depended both on the hybridoma and the strain of origin of the anti-allotype antibody (Table VIII). Furthermore, the two hybridomas specific for the C_{H3} domain and the one reagent specific for the C_{H2} domain of IgG_{2a}^b each inhibited, at a dilution of 1:30, >85% of the binding BAB.14 anti-Id sera to ^{3}H-purified BALB/c anti-CBPC101 antibody, indicating extensive idiotypic sharing between these hybridoma products despite their distinctive specificities.

Overall, these data showed that an IdX or a family of IdX expressed by an anti-(IgG_{2a}^b)-allotype antibody originating from one individual BALB/c mouse or pools of C.AL20, P/J, and DBA/2 anti-allotype sera are also expressed on anti-allotype antibodies of various BALB/c individuals. Furthermore, this IdX was found on anti-allotype antibodies produced in various strains of mice as well as on products of

TABLE VII

Properties of Hybridoma Anti-IgG_{2a}^b Allotype Antibody

Hybridoma	Immunized donors	Specificity of hybridoma product	Specificity for Fc domains	Ig class of hybridoma product	HA titer (log_2)	
					CBPC101 SRBC	C57BL/6 Ig SRBC
10-3.6	CW.B	IA.17	—	G2a	0	0
Ig(1b)5.7.1	BALB/c	IgG_{2a}^b	CH3	G3	12	>12
Ig(1b)3.1	BALB/c	IgG_{2a}^b	CH3	G1	11	10
Ig(1b)2.9	BALB/c	IgG_{2a}^b	CH2	G2a	12	>12
Ig(1b)4.7	SJA	IgG_{2a}^b	Hinge	G1	0	0
S1G.1E	BALB/c	IgG_{2a}^b	ND*	G1	24	24
S1A.1A	BALB/c	IgG_{2a}^b	ND	G1	24	24
BALB/c anti-CBPC101 antibody	BALB/c	IgG_{2a}^b	ND	ND	12	16

* ND, not done.

TABLE VIII

Study of IdX Expression of Hybridoma Anti-IgG_{2a}^b Allotype Antibodies using BAB.14 Anti-Id

Hybridoma number	HI titer (log_2); SRBC coated with				Inhibition of RIA using ^{3}H-labeled			
	BALB/c*	C.AL20*	P/J*	DBA/2*	BALB/c*	C.AL20*	P/J*	DBA/2*
Ig(1b)5.7.1	>8	>8	5	4	100‡	1,000	10	10
Ig(1b)3.1	5	ND§	ND	ND	100	ND	ND	ND
Ig(1b)2.9	4	2	2	>8	>300	10	10	30
Ig(1b)4.7	0	ND	ND	ND	<3	ND	ND	ND
S1G.1E	2	>8	2	>8	3	100	<10	30
S1A.1A	2	>8	4	>8	10	30	<10	100
10-3.6	0	0	0	0	<3	ND	ND	ND

* Anti-CBPC101 antibody.

‡ Results are reported as 1/greatest dilution of hybridoma product which gave >50% inhibition of binding of ^{3}H-anti-allotype antibody to plates coated with a 1:50,000 dilution of BAB.14 anti-(BALB/c anti-CBPC101) antiserum. Dilutions tested were 1:3, 1:10, 1:30, 1:100, 1:300, 1:1,000, and 1:3,000.

§ ND, not done.

hybridomas. The relatedness of the anti-allotype antibodies was further tested by studying the ability of anti-Id sera produced in BAB.14, CXBI, and B.C8 to agglutinate SRBC that were coated with BALB/c, C.AL20, P/J, and DBA/2 anti-CBPC101-purified antibody. The results presented in Table IX show that anti-Id antibodies raised against BALB/c, BALB.B, DBA/2, P/J, and C.AL20 anti-CBPC101 antibodies each interacted, with two exceptions, with BALB/c, DBA/2, P/J, and C.AL20 anti-IgG_{2a}^b allotype antibodies. These observations provide further support for the conclusion that anti-IgG_{2a}^b antibodies produced in various strains express a substantial degree of idiotypic similarity.

Isoelectric Focusing Pattern of Anti-Allotype Antibodies. Anti-CBPC101 antibodies produced by a variety of responding strains showed strikingly similar isoelectric focusing patterns when tested for their ability to bind ^{125}I-Fc fragments of CBPC101 myeloma protein (Fig. 2). The anti-CBPC101 sera of some strains (B.C8, 129, and DBA/2) did differ from other strains in that they lacked antibodies which focused in basic regions of the gel. However, the Fc CBPC101-binding antibodies found in these strains were

TABLE IX

Expression of IdX on BALB/c, C.AL20, P/J, and DBA/2 Anti-Allotype (IgG_{2a}^b) Antibody

Strain in which anti-Id antiserum was produced	Immunogen: anti-CBPC101 produced in	HA titer (log_2); SRBC coated with			
		BALB/c*	C.AL20*	P/J*	DBA/2*
BAB.14	BALB/c	8	>8	>8	>8
CXBI	BALB/c	8	>8	>8	>8
B.C8	BALB/c	7	6	6	6
B.C8	BALB.B	7	6	8	8
B.C8	C.AL20	6	6	6	1
B.C8	P/J	5	0	5	5
B.C8	DBA/2	6	6	6	8

* Anti-CBPC101 antibody.

shared with other responding strains. As anticipated, sera from nonresponder strains (C58 and [BALB/c × C57BL/6]F_1) failed to bind Fc CBPC101.

Discussion

The results reported here indicate that the capacity of mice to produce antibodies specific for allotypic determinants of the IgG_{2a}^b type is associated with the MHC of the responding individuals, with strains of the *b*, *d*, *p*, *q*, *r*, and *s* MHC types being responders. The best evidence for the role of MHC genes in controlling this response is provided by the distinctive response patterns of BALB/c congenic mice; indeed, BALB/c and BALB.B mice respond to CBPC101, whereas BALB.K mice do not. This demonstration of MHC-gene control of responses to the IgG_{2a}^b allotype provides the third example of *Ir* gene control of anti-immunoglobulin responses (8, 9).

Mice of each of the responder MHC haplotypes make anti-allotype antibodies which express IdX determinants found on BALB/c, C.AL20, P/J, and DBA/2 anti-CBPC101 antibodies and identified by BAB.14, CXBI, and B.C8 anti-Id antibodies. Study of these antibodies by isoelectric focusing indicates that the anti-allotype antibody response is of limited heterogeneity.

The expression of an IdX by anti-immunoglobulin antibodies extends the observations of Kunkel et al. (14) that monoclonal human IgM proteins specific for IgG share an IdX and that the light (L) chains of these proteins demonstrate a striking restriction of Ig L-chain variable region (Igl-V) subgroup (15).

The IdX identified on anti-CBPC101 antibodies are expressed on five of six anti-IgG_{2a}^b hybridoma products. These IdX^+ hybridomas are of the IgG_1, IgG_{2a}, and IgG_3 classes, indicating that the expression of the IdX is not limited to Ig of a single class. Furthermore, of the IdX^+ hybridomas, two are specific for a C_{H3}-domain determinant and one for a C_{H2} determinant (13). This demonstration that IdX are expressed on antibodies of apparently different specificities has clear precedence in the work of Oudin and Cazenave on expression of idiotypic determinants on antibodies to distinct fragments of fibrinogen (16) and on sharing of Id by anti-Ova antibodies and by Ig that lacks Ova specificity (17). Similarly, Eichmann et al. (18) have reported that the A5A idiotype is expressed on both anti-streptococcus A carbohydrate antibody and on Ig molecules that are not specific for such determinants. It is a provocative finding

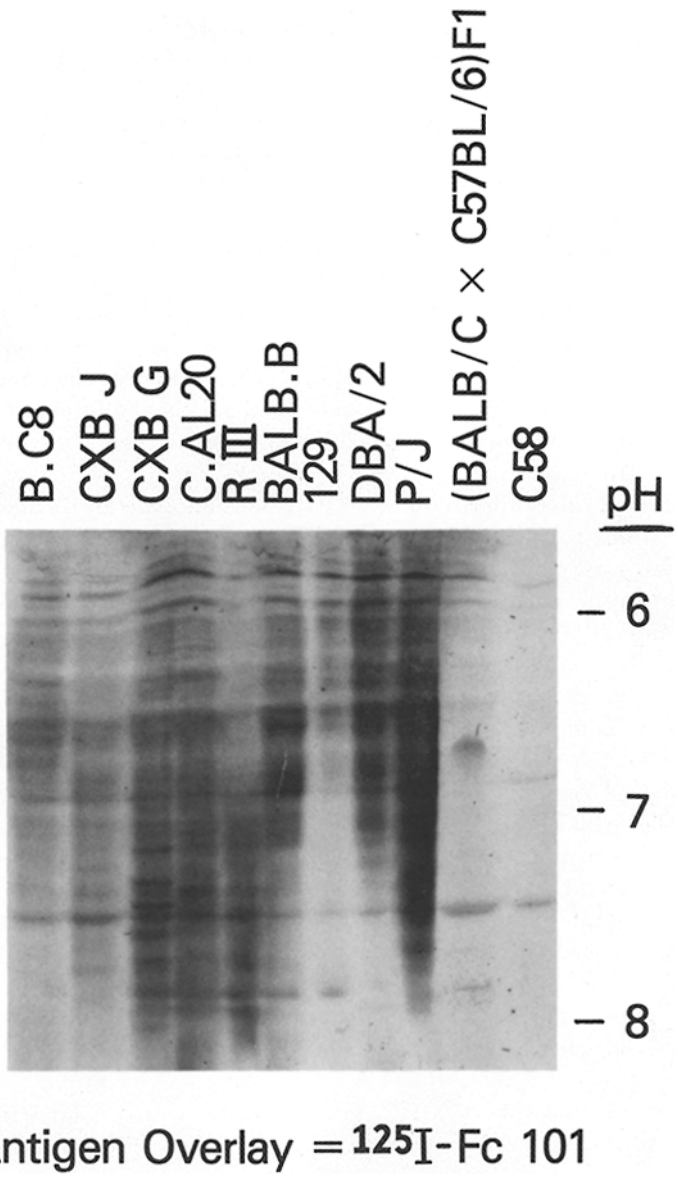

FIG. 2. Isoelectric focusing patterns of anti-CBPC101 antibodies produced by various strains of mice. 10 μl of antiserum (B.C8, CXBJ, CXBG, C.AL20, [BALB/c × C57BL/6]F_1, and C58/J), of a 1:4 dilution of antiserum in normal BALB/c serum (DBA/2 and P/J), or of a 1:8 dilution of antiserum in normal BALB/c serum (RIII and 129) were focused. Gels were overlaid with a ^{125}I-labeled Fc fragment of CBPC101. Radioautographs were exposed for 7 d before development.

that in our studies and that of Cazenave and Oudin (16), antibodies to distinct determinants on the same molecule share idiotypic determinants. The observation that the IdX of the anti-IgG_{2a}^b antibodies are associated with the combining site reinforces the interest of this finding. Possible explanations for such idiotypic sharing by antibodies recognizing distinct specificities on the same molecule is that the V-

region gene for one antibody arose by somatic mutation from the gene for the other (or from a common precursor) and that the presence of antigen selectively stimulated the mutant clone. Alternatively, T lymphocytes or antibody specific for the IdX, generated in response to one determinant on the molecule, may have been indirectly focused upon B lymphocytes specific for other determinants on the same molecule and thus favored the stimulation of IdX^+ clones specific for any determinant on the antigen used for immunization.

The expression of IdX on anti-allotype antibodies has been found in mice of all *Igh-C* types thus far studied, with the exception of the *b* type. The latter, of course, fails to produce anti-IgG_{2a}^b antibody under normal conditions. This sharing of IdX among antibodies of a variety of Igh-C types has previously been noted in the Id of anti-phosphocholine (19), anti-(Glu,Ala,Tyr) (20), and anti-galactan antibodies (21). Recently, anti-Km(1) cryoglobulins in several members of a human family expressed an IdX, the appearance of which was linked neither to their *Gm* type nor their *HLA* type.[3]

The finding of the similarity in anti-IgG_{2a}^b anti-allotype antibodies among a wide variety of strains suggests that the capacity to produce such antibody is a highly conserved function, perhaps reflecting the existence of a germ-line Ig H-chain-variable region (*Igh-V*) gene or family of closely related genes. One may ask why the capacity to produce anti-allotype antibodies should be of sufficient evolutionary significance so that this ability would exhibit the degree of conservation noted. One obvious possibility is that the *Igh-V* genes that encode the combining portion of the H chain may also code for important molecules that normally regulate the expression of given Ig classes. That is, such *Igh-V* genes may code for T cell recognition molecules which, in mice of IgG_{2a}^b type, regulate the expression of B cells capable of producing IgG_{2a}.

In a similar vein, Bellgrau and Wilson (22) have recently reported that $(A \times B)F_1$ rats immunized against T cells from an A donor are resistant to graft-versus-host responses mounted by T cells from A donors and from $(A \times C)$, $(A \times D) \ldots (A \times N)$ F_1 donors. They interpret these results as indicating that there is a limited polymorphism of idiotypic determinants on T cell receptors specific for MHC alloantigens and that the $(A \times B)F_1$ has made a response against idiotypic determinants on A receptors specific for B MHC antigens. Furthermore, the finding that graft-versus-host reaction resistance extends to cells of a series of other MHC types reacting against B antigens suggests that receptors of A, C, D . . . N rats specific for B MHC antigens share Id. The obvious role of MHC gene products as targets for self-recognition in normal immune responses may suggest conservation of responsiveness to MHC antigens, because such responsiveness is critical to the normal function of the immune system. In that respect, one might attempt to draw strong parallels between MHC recognition in cellular interactions and Ig recognition in regulation of class, allotype, and Id of antibody.

Summary

The anti-allotype antibody response to the b allotypic form of IgG_{2a} is regulated by major histocompatibility complex (MHC)-encoded immune response (*Ir*) genes. Mice of *d*, *b*, *p*, *q*, *r*, and *s* haplotypes make a strong anti-allotype response on immunization

[3] Nightingale, S. D., W. B. Bias, N. L. Delancy, and R. P. Pelley. Manuscript submitted for publication.

with the CBPC101 myeloma protein (IgG_{2a}^{b}), whereas mice of the *k*, *m*, *a*, a^1, *u*, and *z* haplotypes made no, or a very poor, response.

All responder strains produce anti-IgG_{2a}^{b} antibodies which share common idiotypes (Id) without relation to the allelic forms of the Ig heavy-chain-constant region genes that the responding mice possess. Isoelectric focusing analysis of the anti-allotype antibodies produced in various strains of mice showed that they are of limited heterogeneity and quite similar from strain to strain. Five out of six hybridoma products with specificity for CBPC101 allotype expressed cross-reactive idiotypes (IdX). Two of hybridoma products expressing IdX identify C_{H3}-domain determinants, and one has been assigned a C_{H2}-domain specificity.

We wish to express our thanks to Ms. Rose Lieberman for many helpful discussions and suggestions.

Received for publication 28 January 1980.

References

1. Cosenza, H., and H. Köhler. 1972. Specific suppression of the antibody response by antibodies to receptors. *Proc. Natl. Acad. Sci. U. S. A.* **69:**2701.
2. Bona, C., R. Lieberman, S. House, I. Green, and W. E. Paul. 1979. Immune response to levan. II. T-independence of suppression of cross-reactive idiotype by anti-idiotype antibodies. *J. Immunol.* **122:**1614.
3. Jacobson, E. A., and L. A. Herzenberg. 1972. Active suppression of immunoglobulin allotype synthesis. I. Chronic suppression after perinatal exposure to maternal antibody to paternal allotype in (SJL × BALB/c)F_1 mice. *J. Exp. Med.* **135:**1151.
4. Eichmann, K., and K. Rajewsky. 1975. Induction of T and B cell immune response by anti-idiotype antibody. *Eur. J. Immunol.* **5:**661.
5. Owen, F. L., S.-T. Ju, and A. Nisonoff. 1977. Presence on idiotype-specific suppressor T cells of receptors that interact with molecules bearing the idiotype. *J. Exp. Med.* **145:**1559.
6. Bona, C., and W. E. Paul. 1979. Cellular basis of regulation of expression of idiotype. I. T-suppressor cells specific for MOPC460 idiotype regulate the expression of cells secreting anti-TNP antibodies bearing 460 idiotype. *J. Exp. Med.* **149:**592.
7. Herzenberg, L. A., K. Okumura, H. Cantor, V. L. Sato, P. W. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. T-cell regulation of antibody responses: demonstration of allotype specific helper T cells and their specific removal by suppressor T cells. *J. Exp. Med.* **144:**330.
8. Lieberman, R., and W. Humphrey. 1971. Association of H_2 types with genetic control of immune responsiveness to IgA allotype in mouse. *Proc. Natl. Acad. Sci. U. S. A.* **68:**2510.
9. Lieberman, R., and W. Humphrey. 1972. Association of H-2 types with genetic control of immune response to IgG (γ_{2a}) allotypes in mouse. *J. Exp. Med.* **136:**1222.
10. Bona, C., R. Hooghe, P. A. Cazenave, C. LeGuern, and W. E. Paul. 1979. Cellular basis of regulation of expression of idiotype. II. Immunity to anti-MOPC460 idiotype antibodies increases the level of anti-trinitrophenyl antibodies bearing 460 idiotypes. *J. Exp. Med.* **149:** 815.
11. Wilder, R., C. C. Yuen, B. Subbarao, V. L. Woods, C. B. Alexander, and R. G. Mage. 1979. Tritium (^3H) radolabeling of protein A and antibody to high specific activity: application to cell surface antigen radioimmunoassays. *J. Immunol. Methods.* **28:**255.
12. Bona, C., J. J. Mond, K. E. Stein, S. House, R. Lieberman, and W. E. Paul. 1979. Immune response to levan. III. The capacity to produce anti-inulin antibodies and cross-reactive idiotypes appears late in ontogeny. *J. Immunol.* **123:**1484.
13. Oi, V., and L. A. Herzenberg. Localization of murine Ig1b and Ig1a (IgG_{2a}) allotypic determinants detected with monoclonal antibodies. *Mol. Immunol.* In press.

14. Kunkel, H. G., V. Agnello, F. G. Joslin, R. J. Winchester, and J. D. Capra. 1973. Cross-idiotypic specificity among monoclonal IgM proteins with anti-γ globulin activity. *J. Exp. Med.* **137:**331.
15. Kunkel, H. G., R. J. Winchester, F. G. Joslin, and J. D. Capra. 1974. Similarities in the light chains of anti-γ globulin showing cross-idiotypic specificities. *J. Exp. Med.* **149:**128.
16. Cazenave, P. A., and J. Oudin. 1973. L'idiotypie des anticorps qui, dans le sérum d'un lapin immunisé contre le fibrinogenè humain, sont dirigés contre deux fragments distinct de cet antigéne. *C. R. Seances Acad. Sci.* **276:**243.
17. Oudin, J., and P. A. Cazenave. 1971. Similar idiotypic specificities in immunoglobulin fractions with different antibody functions or even without detectable antibody function. *Proc. Natl. Acad. Sci. U. S. A.* **68:**2616.
18. Eichmann, K., A. Coutinho, and F. Melchers. 1971. Absolute frequencies of lipopolysaccharide-reactive B cells producing A5A idiotype in unprimed, streptococcal A carbohydrate-primed and anti-A5A idiotype-sensitized and anti-A5A idiotype-suppressed A/J mice. *J. Exp. Med.* **146:**1436.
19. Cancro, M. P., N. H. Sigal, and N. R. Klinman. 1978. Differential expression of an equivalent clonotype among BALB/c and C57BL/6 mice. *J. Exp. Med.* **147:**1.
20. Ju, S.-T., T. Y. Kipps, J. Thèze, and M. Dorf. 1978. Idiotypic analysis of anti-GAT antibodies. I. Presence of common idiotypic specificities in both responder and nonresponder mice. *J. Immunol.* **121:**1034.
21. Mushinski, E. B., and M. Potter. 1977. Idiotypes on galactan binding myeloma proteins and anti-galactan antibodies in mice. *J. Immunol.* **119:**1888.
22. Bellgrau, D., and D. B. Wilson. 1979. Immunological studies of T-cell receptors specific for major histocompatibility complex alloantigens. *J. Exp. Med.* **149:**234.