

Allelic Polymorphism of Murine IgE Controlled by the Seventh Immunoglobulin Heavy Chain Allotype Locus

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Abstract. Two alloantisera against hybridoma-derived IgE detected allotypic determinants expressed on the murine ϵ chain. An antiserum raised in BALB/c mice against monoclonal IgE of C57BL/6 origin reacted exclusively with IgE of strains having Igh-1^b (IgG_{2a}) allotype. The second antiserum, C57BL/6 anti-BALB/c monoclonal IgE, reacted with IgE of strains having Igh-1^a, Igh-1^d, Igh-1^e and Igh-1^j allotypes. The genetic studies of (BALB/c $\times$ C57BL/6)F₁ and backcross F₂ animals indicated that the locus controlling the IgE allotype is linked to the *Igh-1* locus. This was further confirmed by the possession of respective IgE allotypes by Igh-C congenic mice, BALB/c and BAB-14, C3H.SW/Hz and CWB/Hz. Thus, the allotype detected on the ϵ chain is controlled by the seventh murine immunoglobulin allotype locus, and should be designated as the Igh-7 allotype.

Introduction

Allotypic determinants (genetic markers of immunoglobulins) have been determined on most murine immunoglobulin classes except IgG₃ and IgE (Herzenberg and Herzenberg 1978, Green 1979, Lieberman 1978). These de-

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Abbreviations used in this paper: PCA = passive cutaneous anaphylaxis; RID = radioimmunodiffusion; i.p. = intraperitoneally; EA = egg albumin; Igh-C = immunoglobulin heavy chain constant region locus; DNP = 2,4-dinitrophenyl; PBS = phosphate-buffered saline; NMS = normal mouse serum; KLH = keyhole limpet hemocyanin.

terminants are not only useful markers for genetic studies of immunoglobulins, but also provide important information on the origin of the polymorphism of immunoglobulin genes. There has been no description so far of the genetic polymorphism of the IgE molecule, probably due to the low serum concentration of IgE in normal animals and to the lack of murine IgE myeloma. This difficulty was, however, recently overcome by the establishment of hybridomas which produce a large quantity of monoclonal IgE (Böttcher et al. 1978, Böttcher et al. 1980, Eshhar et al. 1980) and enable us to produce alloantisera which detect allotypic determinants present only on IgE. By the sensitive assays of inhibition of passive cutaneous anaphylaxis and radioimmunoassay, we were able to determine at least two allotypic determinants allelically expressed on murine ϵ chains. The genetic studies indicated that the allotype is clearly linked to the Igh-1 (IgG_{2a}) allotype, and thus it is designated Igh-7.

Materials and Methods

Animals. Inbred BALB/cAnN, AKR/J, C3H/HeN, and A/J mice were purchased from Omura Experimental Animal Company (Sagamihara, Kanagawa, Japan). C57BL/6CrSlc mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Shizuoka, Japan). Strains of SJL/J, C3H.SW/Hz, C57BL/6J, and BAB-14 mice were originally provided by Dr. L. A. Herzenberg, Department of Genetics, Stanford University School of Medicine, Stanford, California. B10.BR mice were gifts from the Department of Experimental Animal Supply, Research Institute of Medical Science, University of Tokyo, Tokyo, Japan. (BALB/c $\times$ C57BL/6)F₁ hybrid and backcross animals were produced in our own animal facility. We also used two additional colonies of BALB/c and C57BL/6J mice which were purchased from Cumberland View Farms, Clinton, Tennessee, and from Jackson Laboratory, Bar Harbor, Maine, respectively.

Monoclonal IgE from hybridomas. Monoclonal IgE of C57BL/6 (*Igh-C^b/Igh-C^b*) origin with specificity against a hapten, 2,4-dinitrophenyl (DNP), was obtained from the culture supernatant of a hybridoma kindly provided by Dr. Zelig Eshhar, Weizmann Institute, Rehovot, Israel. IgE was purified by adsorption to and elution from a column of DNP-Sepharose CL4B (Pharmacia, Uppsala, Sweden). The elution was performed with 0.05M DNP-L-glycine, pH 7.4, followed by extensive dialysis against phosphate-buffered saline (PBS), pH 7.4. The purity of IgE was confirmed by polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS).

Two IgE-producing monoclonal hybridomas originated from BALB/c (*Igh-C^a/Igh-C^a*), one having specificity against egg albumin (EA) (clone 14-205) and the other against DNP (clone 52), were kindly provided by Dr. I. Böttcher (Research Laboratories Shering AG, Berlin, Federal Republic of Germany). DNP-specific monoclonal IgE from clone 52 was purified from the ascitic fluid of BALB/c mice transplanted i.p. with line 52 cells.

Anti-allotype sera. Standard antisera against IgG_{2a} allotypes (anti-Igh-1^a and anti-IgG-1^b) were kindly supplied by Dr. L. A. Herzenberg. The antiserum, putatively directed against allotypic determinants on IgE of the C57BL/6 strain, was produced at Tokyo University by immunizing BALB/c (*Igh-C^a/Igh-C^a*) mice with monoclonal IgE. One billion heavily DNP-coupled *Bordetella pertussis* organisms were covered with monoclonal anti-DNP IgE antibody, and were repeatedly injected intraperitoneally (i.p.) into BALB/c mice on days 1, 3, 5, 22, 26, 50, and 57. Mice were bled a week after the last immunization. The sera were pooled and kept at -80° C until use. The second antiserum, putatively directed against allotypic determinants on IgE of BALB/c origin, was produced at New York University by immunizing C57BL/6 (*Igh-C^b/Igh-C^b*) mice with monoclonal IgE from line 52. Ten micrograms of affinity-purified, anti-DNP IgE was injected i.p. with Freund's complete adjuvant (total volume 0.5 ml) into 8-week-old female C57BL/6 mice. These antisera were exchanged between Tokyo and New York via air mail, and reciprocal testing was made in each laboratory.

Guinea pig anti-IgE. The antiserum was raised by two immunizations with 50 µg of purified IgE in Freund's complete adjuvant with a 2-week interval. The antiserum was absorbed with purified mouse IgG to remove anti-Fab activity. The absorbed antiserum did not develop precipitin lines with mouse IgA(MOPC 315), IgM(HPC 76 myeloma), IgG_{2a}(X5563), IgG_{2b}(MOPC 11), and IgG₁(MOPC 31C) in an Ouchterlony plate, while a single clear precipitin line was formed with all of the monoclonal IgE. The antiserum also developed a single precipitin line with sera of mice infected with *Nippostrongylus brasiliensis* (see below) but not with normal mouse serum in immunoelectrophoresis.

Mouse antisera containing IgE antibodies against EA. Mice were immunized i.p. with 1 µg of EA (recrystallized five times, ICN Pharmaceutical Inc., Cleveland, Ohio) in 4 mg of alum (Ovary et al. 1978). Animals were boosted with 1 µg of antigen in alum during the 4th week and bled 5 days later. For C3H/HeN, AKR/J and B10.BR (*H-2^k*) mice, 50 µg of EA for priming and boosting were required to induce an adequate IgE antibody response against EA (Levine and Vaz 1970).

Passive cutaneous anaphylaxis (PCA). The detection of antibodies of the IgE class was carried out as described by Ovary and co-workers (1975). Pairs of Sprague Dawley rats were sensitized by intradermal injections of serial dilutions of anti-EA sera, and were challenged 2 h later by intravenous injection of 1 mg EA with 1 ml of 1% Evans blue solution in saline. The reaction was observed on the internal side of the skin 30 min after the challenge. Neutralization of the PCA activity with allotype-specific antibodies was carried out by mixing anti-EA sera from various strains with the anti-allotype serum at a ratio of 1:10 or 1:15. After incubation at 4°C for 24 h the mixture was centrifuged at 40 000 g for 1 h, and the supernatant was diluted in PBS to test the residual PCA activity. Normal mouse serum from BALB/c or C57BL/6 was used as a control to exclude possible nonspecific inhibition.

Infection of mice with Nippostrongylus brasiliensis. Mice were infested with *N. brasiliensis* larvae kindly provided by Dr. N. Watanabe (Jikei University School of Medicine, Tokyo, Japan). Third-stage larvae (750/mouse) were injected subcutaneously (Ogilvie 1967), and the sera were obtained 2 weeks after infection. This procedure resulted in an increase in the serum IgE concentration enough to develop a precipitin line with anti-IgE serum in double immunodiffusion.

Radioimmunoassay. A modified method of Ishizaka and Ishizaka (1967) was used to detect IgE allotype. The radiolabelled anti-allotype was prepared as follows: 0.5 ml of anti-IgE allotype serum was passed through an immunoadsorbent column of monoclonal IgE (total bed volume, 0.5 ml). After washing, antibodies were eluted with 0.1 M glycine-HCl buffer, pH 3.0, and were concentrated after immediate neutralization. They were labelled with ¹³¹I by the method of Hunter and Greenwood (1962). Sera from *N. brasiliensis*-infected mice were reacted with guinea pig anti-IgE to develop a visible precipitin line in Ouchterlony plates. Plates were washed and then rinsed with radiolabelled, purified allotype-specific antibodies. After washing and drying, plates were contacted with X-ray film to develop autoradiogram.

Results

Neutralization of PCA activity by anti-allotype serum. Antisera from various strains of mice having high PCA activity against EA were mixed with anti-allotype serum or normal BALB/c serum. After incubation and centrifugation, the residual PCA activity was determined. Figure 1 shows a representative result which demonstrates complete inhibition of PCA reaction of C57BL/6 antiserum but not of BALB/c by the putative anti-IgE allotypes produced in BALB/c mice. Significant reduction of the PCA titer was consistently observed when the anti-EA sera from C57BL/6, CWB/H₂, and B10.BR were mixed with the anti-allotype but not with NMS (Table 1). No decrease in the PCA titer was observed when anti-EA sera from A/J, C3H.SW/H₂, C3H/HeN, and AKR/J were mixed with the same anti-allotype serum. Thus, the anti-allotype serum used in this experiment neutralized PCA

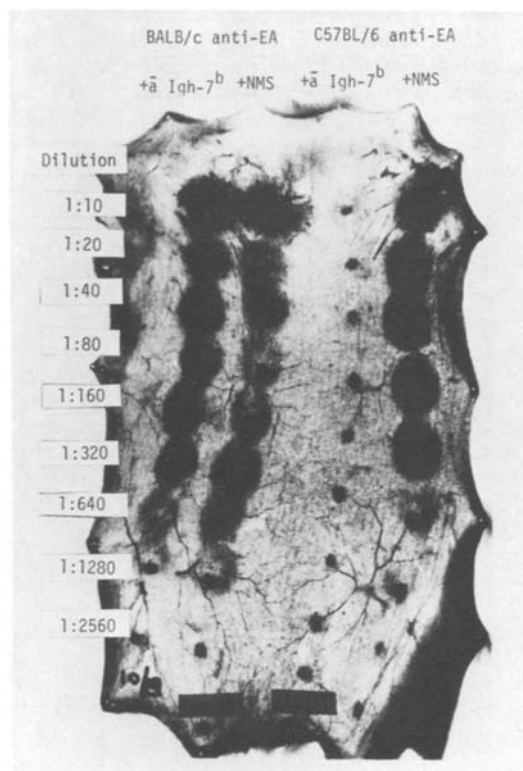


Fig. 1. Inhibition of PCA activity of C57BL/6 but not of BALB/c sera by the anti-allotype serum raised in BALB/c mice. Anti-EA antisera from both strains were mixed with normal mouse serum or anti-Igh-7^b ($\bar{a}$ Igh-7^b) antiserum. After 24 h of incubation and centrifugation, the residual PCA activity of the mixture was tested. Note the complete inhibition of PCA reaction of C57BL/6 but not of BALB/c antisera by the allotype-specific antibodies.

activity of the strains having the Igh-1^b allotype but not others (Igh-1^a for BALB/c, Igh-1^j for C3H/HeN and C3H.SW/Hz, Igh-1^e for A/J, and Igh-1^d for AKR/J).

Using the antisera produced in C57BL/6 mice against IgE from cell line 52 (an IgE-producing hybridoma of BALB/c origin) a significant reduction of PCA titer was observed with anti-EA sera from BALB/c (Igh-1^a), AKR (Igh-1^d), A/J (Igh-1^e), and C3H.SW/Hz (Igh-1^j) (Table 1). No decrease in PCA titer was observed with antisera of Igh-1^b strains, i.e., C57BL/6 and CWB/Hz. Since C3H.SW/Hz (Igh-1^j) and CWB.Hz (Igh-1^b) are congenic for *Igh-C* loci, it was concluded that these two alloantisera detected allotypic determinants controlled by *Igh-C* loci allelically expressed on IgE of different strains of mice. It is also apparent that the detected allotypic determinants of IgE are common or cross-reactive at least among Igh-1^a, Igh-1^d, Igh-1^e, and Igh-1^j strains.

It was also confirmed that the allotypic determinant detected by the antiserum against one hybridoma (line 52) was able to react with IgE of another hybridoma with anti-EA activity (line 14-205). Figure 2 shows that a monoclonal IgE antibody with specificity for EA was absorbed by the anti-allotype serum prepared against line 52 monoclonal IgE specific for DNP. In addition, the same alloantiserum could neutralize the skin-sensitizing activity of KLH-specific antibody of BALB/c but not of C57BL/6 mice (Fig. 2). All the above data indicate the existence of at least two allotypes showing the genetic polymorphism of murine IgE (Igh-7), one for strains having Igh-1^b and the other shared by Igh-1^a, Igh-1^d, Igh-1^e, and Igh-1^j strains.

Table 1. Neutralization of sensitizing activity of mouse antisera against EA by anti-Igh-7 antisera*

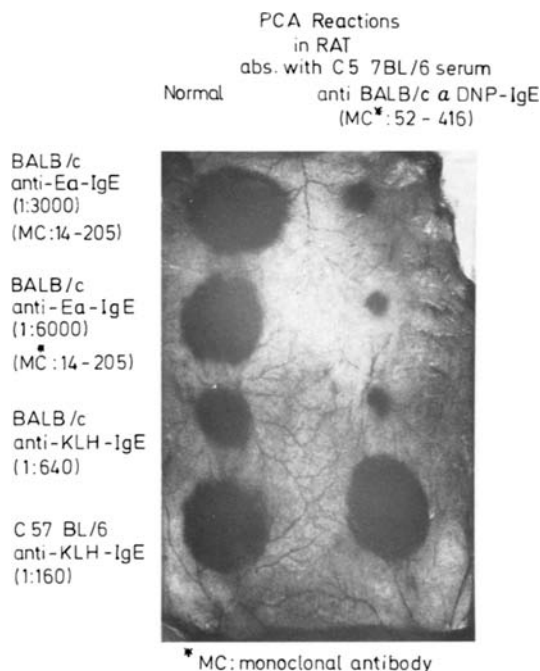
Strain	H-2 haplotype	Igh-1 allotype	Reciprocal PCA titer against EA after absorption with			
			NMS(BALB/c) [†]	Anti-Igh-7 ^b	NMS(C57BL) [†]	Anti-Igh-7 ^a
BALB/c	<i>d</i>	<i>a</i>	2560	2560	2400	≤ 300
C57BL/6	<i>b</i>	<i>b</i>	1280	< 40	2400	2400
C3H.SW/Hz	<i>b</i>	<i>j</i>	320	320	480	≤ 60
CWB/Hz	<i>b</i>	<i>b</i>	160	40	480	480
C3H/HeN	<i>k</i>	<i>j</i>	1280	1280	ND	ND
AKR/J	<i>k</i>	<i>d</i>	320	320	240	≤ 60
A/J	<i>a</i>	<i>e</i>	640	640	480	≤ 60
B10.BR	<i>k</i>	<i>b</i>	320	< 40	ND	ND

* Antisera from different strains containing anti-EA of the IgE class were mixed with anti-allotype antisera at a ratio of 1:10 (anti-Igh-7^b) or 1:15 (anti-Igh-7^a). After a 24 h incubation at 4°C and centrifugation at 40 000 G the residual PCA activity of the supernatant was determined.

[†] Normal mouse serum (NMS) obtained from either BALB/c or C57BL/6 was used as control. ND = not determined.

Direct demonstration of allotypic determinants on IgE by radioimmunodiffusion (RID). The results of RID are demonstrated in Figure 3a and 3b. In Figure 3a, visible precipitin lines with guinea pig anti-IgE and *N. brasiliensis*-infested sera are developed in all the mouse strains tested. Whereas the precipitin lines developed with the sera from those having the Igh-1^b allotype (C57BL/6, CWB/Hz, BAB-14, B10.BR, and SJL/J) bound radioactive allotype-specific antibodies produced

Fig. 2. Inhibition of PCA activity of BALB/c but not of C57BL/6 antisera with specificity for EA or KLH raised in C57BL/6 mice. Anti-EA or anti-Igh-7^a serum. After 24 h of incubation and centrifugation, the residual PCA activity of the mixtures was tested. Note the complete inhibition of PCA reaction of BALB/c but not of C57BL/6 antisera by the allotype-specific antibodies.



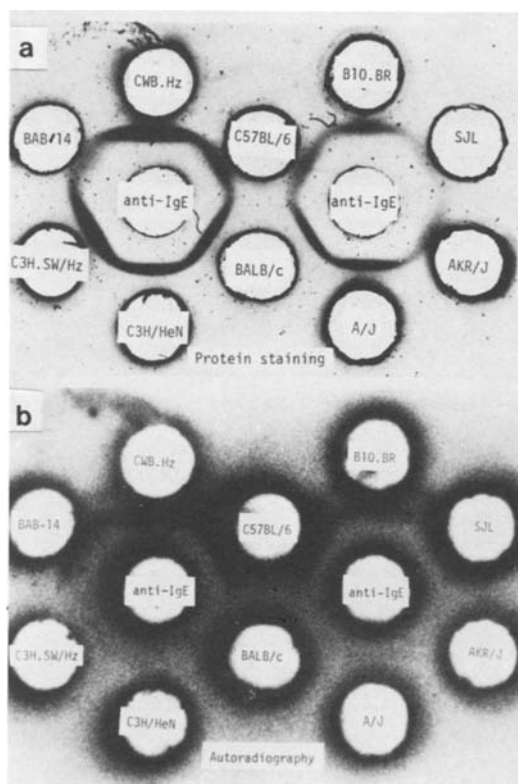


Fig. 3a and b. Radioimmunodiffusion analysis of (a) stained slides, (b) autoradiograms. Guinea pig anti-mouse IgE serum was placed in the center wells. Sera from *N. brasiliensis*-infested mice were placed in peripheral wells. After formation of precipitin lines (a), the plates were washed and purified ^{125}I IgE- 7^b -specific antibodies were placed on them. IgE from *Igh-1^b* strains contained radioactive antibodies (b).

against IgE of C57BL/6 (anti-Igh- 7^b), those developed with other *Igh-1* allotypes showed no corresponding radioactive lines (Fig. 3b, and summarized in Table 3). Again the strain distribution of the binding of radioactive allotype-specific antibodies was identical to that of the *Igh-1^b* allotype.

Genetic analysis. In order to analyze the linkage of the IgE allotype to *Igh-1^b*, backcross studies were carried out. (BALB/c $\times$ C57BL/6) F_1 males were mated with female BALB/c mice. The F_2 mice thus obtained were infested with *N. brasiliensis* to obtain high serum levels of IgE. *Igh-1* homozygotes (*Igh-1^a/Igh-1^a*) and heterozygotes (*Igh-1^a/Igh-1^b*) were determined by testing their sera with anti-Igh-1 in double immunodiffusion. The sera obtained 2 weeks after the infection were subjected to RID to determine the IgE allotype as described above. F_2 mice segregated into *Igh-1^a/Igh-1^a* homozygotes and *Igh-1^a/Igh-1^b* heterozygotes at a ratio of approximately 1:1 (Table 2). IgE allotype detected by radioactive anti-Igh- 7^b was found exclusively in *Igh-1^a/Igh-1^b* heterozygotes. None of the *Igh-1^a/Igh-1^a* homozygotes had this allotype, indicating the strong linkage of the IgE allotype with the *Igh-1* allele.

Evidence that the allotypic determinants are expressed uniquely on IgE. None of the available anti-allotype sera directed against *Igh-1^b*, *Igh-5^b*, and *Igh-4^b* could

Table 2. Genetic linkage between Igh-1^b and Igh-7^b as analyzed by RID with radioactive Igh-7^b-specific antibodies*

Igh-1 phenotype of backcross F ₂ [†]	Number of mice	Positive reaction with anti-Igh-7 ^b [‡]
a/a	16	0/16
a/b	15	15/15

* Female BALB/c mice mated with male CBF₁; F₂ mice were infested with *N. brasiliensis* and sera were obtained 2 weeks after infection.

[†] Tested by Ouchterlony analysis with anti-Igh-1^a and anti-Igh-1^b serum.

[‡] Binding to radiolabelled anti-Igh-7^b.

neutralize the PCA activity of the C57BL/6 antiserum. The radioactive anti-Igh-7^b antibody did not bind to the precipitin lines developed with anti-isotype sera anti-IgM and anti-IgG. The results indicate that the allotypic determinants detected with the alloantiserum against monoclonal IgE are expressed only in the IgE class.

Discussion

The alloantiserum produced by immunization of BALB/c mice with IgE from the hybridoma supernatant of C57BL/6 origin was found to inhibit the skin-sensitizing (PCA) activity of IgE antibody from mouse strains having the Igh-1^b allotype but not from those having different Igh-1 allotypes. Conversely, alloantiserum of C57BL/6 immunized with BALB/c monoclonal IgE could neutralize PCA activity of the strains having Igh-1^a, Igh-1^d, Igh-1^e, and Igh-1^j but not Igh-1^b. The results indicate that allotypic determinants are present on the murine IgE molecule. This was confirmed by the direct demonstration of binding of radioactive alloantibodies to precipitin lines developed with IgE from strains having Igh-1^b but not those having other Igh-1 allotypes. The analysis with Igh-C congenic mice (BALB/c and BAB-14, C3H.SW/Hz and CWB/Hz), as well as backcrossed F₂ mice indicated that the determinants are genetically linked to Igh-1^b. Table 3 summarizes the parallel distribution of this allotype among strains as examined by two different methods. The present results indicate that the putative allele for IgE polymorphism is linked to the *Igh-1* allele, and that at least two alleles can be distinguished by our anti-allotype sera. Thus, our conclusion is that this allotypic determinant on IgE linked to Igh-1^b should be called Igh-7^b and those possessed by the Igh-1^a, Igh-1^d, Igh-1^e, and Igh-1^j strains should be named Igh-7^a according to the nomenclature of murine Ig allotypes (Green 1979).

A few points should be considered for the definition of this IgE allotype. In our present experiments, we have used monoclonal IgE to produce allotype-specific antibodies. The antiserum should also contain anti-idiotypic activity. The antiserum used in the present experiments did in fact, contain some but appreciable idiotypic-specific antibodies which can only react with homologous but not other hybridoma-derived IgE. However, the antiserum could neutralize IgE antibodies

Table 3. Correlated distribution of the Igh-7^b allotype among strains as examined by radioimmunodiffusion and PCA inhibition

Strain	H-2 haplotype	Igh-1 allotype	Igh-7 ^b detected by	
			RID*	PCA inhibition
BALB/c	<i>d</i>	<i>a</i>	—	—
BAB-14	<i>d</i>	<i>b</i>	+	ND
C3H.SW/Hz	<i>b</i>	<i>j</i>	—	—
CWB/Hz	<i>b</i>	<i>b</i>	+	+
SJL	<i>s</i>	<i>b</i>	+	ND
C57BL/6	<i>b</i>	<i>b</i>	+	+
AKR/J	<i>k</i>	<i>d</i>	—	—
C3H/HeN	<i>k</i>	<i>j</i>	—	—
A/J	<i>a</i>	<i>e</i>	—	—
B10.BR	<i>k</i>	<i>b</i>	+	+

* Radioimmunodiffusion with Igh-7^b-specific antibodies. ND = not determined.

against unrelated antigen (KLH) as well as monoclonal IgE specific for EA, and could react with IgE produced by polyclonal stimulation by the infestation with *N. brasiliensis*. These results exclude the possibility that the neutralization of PCA activity and the binding to the IgE precipitin line are due to the presence of cross-reactive, idiotype-specific antibodies.

The second question is whether the neutralization of PCA reaction may be due to the nonspecific competitive inhibition of sensitization by "normal" IgE in the inhibitory serum as reported by Ovary (1964). In our experiments, however, no inhibition of PCA was observed with a high concentration of normal serum from both BALB/c and C57BL/6 mice, the same strains used as producers of anti-allotype sera. Since IgE is bound to mast cells through the Fc portion of its molecule, the property of the antiserum to inhibit the sensitizing capacity of antisera from Igh-1^b mice but not from mice having other Igh-1 allotypes is strong evidence for its anti-allotypic activity.

It is also interesting to note that the strain distribution of Igh-7^b strictly follows the inheritance pattern of the Igh-1^b allotype, and that the Igh-7^a allotype shows cross-reactivity of Igh-1^a, Igh-1^d, Igh-1^e, and Igh-1ⁱ among the mouse strains tested. This distribution pattern is analogous to that of Igh-4 in which most mouse strains are divided into Igh-4^a and Igh-4^b. It is also known that there are cross-reactive allotypic determinants on IgG_{2a} which are shared by strains having different *Igh-1* alleles (e.g., antigenic specificities 6, 7, 8, and 29). It is safe to conclude that IgE allotypes are controlled by at least two alleles but further polymorphism could be determined by the production of alloantisera with different strain combinations.

The discovery of murine IgE allotypic determinants will provide a new approach to the study and comprehension of the genetic factors involved in the control of the production of IgE antibodies. It may be particularly important for the understanding of the heredity of some allergic diseases. In addition, allotype suppression of IgE may have potential application for the prevention of hereditary allergic diseases.

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