Immunogenetics 9: 455–463, 1979

Immunogenetics

A Cross-Over Chromosome Combining *Ig* Heavy Chain Genes of Two Mouse Strains

Cornelia Kolb[1], E. Weiler[1], I. Seppälä,[2,*] K. Eichmann[2], M. Kaartinen[3], J. Pelkonen[3], K. Karjalainen[3], and O. Mäkelä[3]

[1] Fachbereich Biologie, University of Konstanz, D-775 Konstanz, Federal Republik of Germany. [2] Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum Heidelberg, D-6900 Heidelberg, Federal Republic of Germany. [3] Department of Bacteriology and Immunology, University of Helsinki, Haartmaninkatu 3, 00290 Helsinki 29, Finland

Abstract. Two congenic strains of mice were bred in Konstanz that bear the *Ig* heavy chain allotype gene of the C57BL/6 (*Ig-1*^{b}) in a BALB/c background genome. One line (CB-8K) underwent eight backcross generations to BALB/c before sister-brother mating was initiated. For the other line (CB-16KN) backcrossing was continued for eight further generations, then a homozygous *Ig-1*^{b}/*Ig-1*^{b} strain was produced by sister-brother mating.

Both lines were tested for four V_H markers of the BALB/c and one of the C57BL parent. The CB-16KN strain expressed the C57BL marker V_HNP^b together with the C57BL allotype marker, and failed to express the three BALB/c markers, V_HDEX, V_HS117, $V_H$phOx and V_HNP^a. It thus resembled the CB-20 strain.

Strain CB-8K expressed the V_HNP^b marker of C57BL but also all the four BALB/c markers that were tested. The strain appeared more heterogeneous than the CB-16KN strain, and a subline was bred from two exceptional mice that did not express the V_HNP^b marker. This subline (CB-8KN) expressed the BALB/c marker V_HNP^a regularly, and was negative for the V_HNP^b marker. It thus resembles the BAB-14 line.

The crossing over event thus must have happened in one of the two meioses, which led to the CB-8K line. As BAB-14 is derived in an analogous manner to a branch of the backcross of Potter and Lieberman, which ended up in CB-20, the unexpected finding is discussed that two independent crossing over events (in CB-8KN and BAB14) within the *Ig* heavy chain gene region have taken place at approximately the same stage of two breeding programs.

* Recipient of an EMBO fellowship during part of the study

0093-7711/79/0009/0455/$ 1.80

Introduction

Several V_H genes, presumably *Ig* heavy chain variable region gene loci, have been identified in the mouse. These markers are linked to a tight cluster of allotype loci (*Ig-1*, *Ig-2*, *Ig-3*, *Ig-4*, *Ig-5* and *Ig-6*) called the allotype cluster. These are presumambly genes for the immunoglobulin heavy chain constant regions. No genetic recombination events have been recorded within the allotype cluster but the V_H markers occasionally recombine with the allotype cluster or with each other. Some of the recombinant chromosomes have been found from individual backcross mice (Riblet 1977, Eichmann 1975), others from recombinant inbred strains (Berek et al. 1976, Lieberman et al. 1976), and others still in congenic strains that carry an Ig allotype in a new background genome (Fathman et al. 1977, Riblet et al. 1975 b).

A congenic strain was bred in the Konstanz laboratory which carries the Ig-1 allotype b in the BALB/c background. A homozygous *b/b* line was produced after eight and another after 16 backcross generations, they are called strains CB-8K and CB-16KN. Strain CB-8K was found to carry the V_HDEX gene (Blomberg et al. 1972) of the BALB/c strain which suggested that it has a recombinant chromosome. We therefore decided to test both strains for five different V_H markers, and report the results below.

Materials and Methods

Mice. Seven strains of mice were studied, and all were produced in the animal colonies of either the Konstanz or the Helsinki laboratory. The breeding nuclei of strains C57BL/6 and BALB/c were obtained from the Jackson laboratories, the nucleus of the CB-20 from Dr. M. Potter, the National Institutes of Health, and the nucleus of the BAB-14 strain from Dr. Leonore Herzenberg, Stanford University. Strains CB-8K, CB-8KN and CB-16KN were bred as follows: F_1 (BALB/c-AnNIcr × C57BL/6-JNIcr) males were mated to BALB/c females, the litters were tested for allotype b by double diffusion in gel at 8 weeks of age, and allotype b-positive males were then mated to BALB/c females. Eight backcross generations were produced in this manner, whereby only one single mating pair served as progenitors for the next generation. By one brother-sister mating of $Ig\text{-}1^a/Ig\text{-}1^b$ heterozygous mice from the eighth backcross homozygous $Ig\text{-}1^b/Ig\text{-}1^b$ mice were produced, the progenitors of the CB-8K line. The backcrossing was continued for eight further generations in a branch line, then the CB-16KN line was produced, again by one single brother-sister mating. The mice which were used in this study derived from the fourth, fifth and sixth sister-brother generation of the CB-8K line, and from the third generation of the CB-16KN line.

Strain CB-8KN is a subline of CB-8K. A male and a female negative for the V_HNP^b marker (exceptional individuals) were bred, and from their progeny a random sister-brother mating was continued.

The genetic markers. Allotyping of the CB-8K and CB-16KN strains was carried out in Konstanz and checked in Helsinki. Strain CB-8KN was allotyped in Helsinki. More than 30 animals were tested by anti-a and anti-b (double diffusion in gel), and all were positive for b but negative for a.

Dextran. Mice were immunized with 10 μg of α 1–3 dextran B 1355 S i. p., not before they were 2 months old, and bled 7 days later. The serum titers were tested by hemagglutination (HA) of dextran sensitized sheep red blood cells in polystyrene V-microtiter plates using Takatsy loops. The diluent was phosphate buffered saline (PBS) containing 1 mM EDTA, 1 mM NaN_3 and 2% fetal calf serum (PBS-EAS) Leon et al. 1970, Weiler et al. 1977).

To determine the proportion of J558 idiotype among antibodies to dextran, A/J anti-J558 (1/100 in PBS-EAS) was used as diluent for the test serum in a HA-titration. This was compared to parallel dilutions of the test serum in PBS-EAS alone (Weiler et al. 1977).

S117. The mice were immunized with Group A streptococcal vaccine as described earlier (Eichmann 1972). The sera obtained from the mice were tested for S117 idiotype content by radioimmune inhibition assay using anti-S117 idiotype sera prepared in guinea pigs as described earlier (Eichmann and Kindt 1971, Berek et al. 1976).

phOx. Mice were immunized with 2-phenyloxazolone coupled to chicken serum albumin and their sera were tested as described previously (Mäkelä et al. 1978). The sera that were tested in this study were bled 20 days after the first injection of antigen.

NP. Mice were immunized with 100 μg of 3-nitro-4-hydroxyphenylacetyl (NP) coupled to fowl gamma globulin, bled 17 days later, and their anti-NP antibodies were tested for idiotypes NP-b and NP-a as previously described (Karjalainen and Mäkelä 1978, Karjalainen 1979).

Results

V_HDEX

The presence of this marker was decided on the basis of the titer distributions of anti-dex antibodies in the strains to be typed on the 7th day of the primary response. The presence of the V_HDEX marker is indicated by a strong response and its absence is associated with a weak response (Riblet et al. 1975 a). In addition, BALB/c, C57BL and CB-8K antibodies were titrated in the presence of anti-J558 idiotype antibodies, which reduce the titer of antibodies positive for the J558 idiotype (Riblet et al. 1975 a). Data in Figure 1 indicate that CB-8K mice had high titers like BALB/c mice but CB-16KN mice had low titers like C57BL mice. The titers of six out of eight CB-8K mice were reduced by anti-idiotype to one-half which confirmed that the CB-8K strain had the V_HDEX marker (Table 1). The two mice that did not show reduction by anti-J558 antiserum could indicate that two Ig-1 linkage groups may still segregate on CB-8K mice (see below).

V_HS117

The data in Table 2 show that the CB-16KN strain is phenotypically like C57BL/6, whereas five of the seven CB-8K mice expressed S117 idiotypic determinants clearly like BALB/c mice. Two CB-8K mice were outside the range of BALB/c mice. The two S117-negative mice can be interpreted on the one hand as occasional mice in which the marker is lacking because of incomplete penetrance, since in BALB/c mice, too, there is great variability with respect to S117 expression. On the other hand, it is possible that two different *Ig-1* complexes are segregating in CB-8KN mice, only one of them carrying V_HS117 (see below).

$V_H$phOx

The presence or absence of this marker was judged by idiotype analysis. The results in Table 3 confirm our earlier findings that strains C57BL/6 and CB-20 produce idiotype-negative antibodies but strains BALB/c and BAB-14 produce idiotype-positive anti-phOx antibodies. All seven CB-16KN sera were as negative as C57BL and CB-20 sera. Of the fourteen CB-8K sera, two were negative and the remaining 12 were clearly positive. Their antisera had much less idiotype than BALB/c sera or

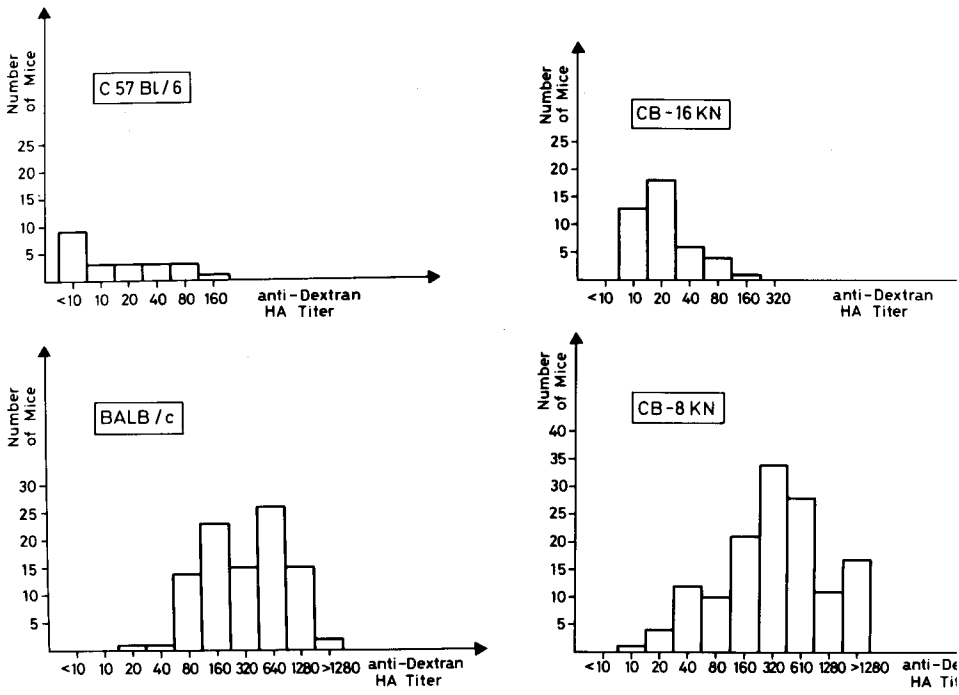

Fig. 1. Distribution of anti-dextran hemagglutination titers in four strains of mice. Mice were immunized with 10 μg α→(1→3) Dextran B 1355 S i. p. at the age of 8 weeks and bled 7 days later. Total numbers of mice were: 138 CB-8K, 42 CB-16 KN, 97 BALB/c, 22 C57BL/6.

Table 1. Effect of A/J anti-idiotype antiserum (anti-J558) on anti-dextran titers of various mouse strains

		Hemagglutination titer without anti-J558	with anti-J558	= Reduction
BALB/c mouse	1	320	80	4×
	2	640	160	4×
	3	160	40	4×
	4	160	80	2×
	5	320	80	4×
CB-8K mouse	1	160	80	2×
	2	80	40	2×
	3	160	40	4×
	4	160	40	4×
	5	80	80	–
	6	40	20	2×
	7	40	40	–
	8	80	20	4×
C57BL/6 mouse	1	10	10	–
	2	< 10	< 10	–
	3	10	10	–
	4	80	80	–

Sera were taken on day 7 of the primary response.

Table 2. Expression of S117 idiotype in antisera to group A streptococcal carbohydrate of various strains of mice

		Reciprocal of the serum dilution that causes 50% inhibition of idiotype binding		
		1st	2nd	3rd bleeding*
CB-16KN mouse	1	75	714	285
	2	100	125	215
	3	50	55	125
	4	63	155	75
	5	70	429	70
	6	120	200	175
	7	105	50	145
CB-8K mouse	1	13000	24000	11000
	2	775	355	549
	3	195	526	380
	4	6700	20400	5900
	5	3800	12200	5500
	6	6000	15600	5100
	7	6000	10400	6200
C57BL/6†		220		
BALB/c		1200–17800‡		
BAB-14‖		1600		

* Three bleedings were obtained from the mice at days 18, 33 and 43 of immunization with group A streptococci.
† An individual hyperimmune mouse.
‡ Range obtained in several experiments.
‖ Pool of 11 sera obtained at day 17 of immunization with group A streptococci.

Table 3. Expression of the strain-specific idiotype in anti-phOx sera of different types of mice

	Reciprocal of the serum dilution that causes 50% inhibition of idiotype binding
Pool of 8 C57BL/6 sera	>100
Pool of 8 CB-20 sera	>100
CB-16KN sera (individuals)	>100 >100, >100, >100 >100, >100
CB-8K sera (individuals)	>100 >100, 1000, 1100, 1300, 1400, 1700, 2300, 2400, 2500, 3000, 3000, 3100, 4000
Pool of 22 BAB-14 sera	9000
Pool of 19 BALB/c sera	12000

Sera were taken on day 20 of the primary response.

BAB-14 sera. This is partly due to lower antibody concentrations in the CB-8K sera but another possible explanation is heterozygosity (see below). The slopes of CB-8KN sera were indistinquishable from the BALB/c slope. These sera appear to have the same idiotype as BALB/c sera although in lower amounts.

Table 4. Content of idiotype NP-b and idiotype NP-a in anti-NP sera of different types of mice. Sera were taken on day 17 of the primary response

		Reciprocal of the serum dilution that causes 50% inhibition of idiotype binding	
		NP-b	NP-a
Pool of 15 C57BL/6 sera		4200	< 100
Pool of 10 CB-20 sera		3200	< 100
CB-16KN mouse	1	4700	< 100
	2	3500	< 100
	3	3100	< 100
	4	2800	< 100
	5	2800	< 100
CB-8K mouse	1	2900	< 100
	2	2700	< 100
	3	2700	1700
	4	2500	1600
	5	1800	1100
	6	2100	1400
	7	2700	1200
	8	700	800
	9	< 100	1600
	10	< 100	2700
	11	< 100	1800
CB-8KN mouse	1	< 30	1700
	2	< 30	1500
	3	< 30	2100
	4	< 30	1000
	5	< 30	1800
	6	< 30	2700
	7	< 30	2200
	8	< 30	2400
	9	< 30	1700
Pool of 10 BAB-14 sera		< 100	2800
Pool of 8 BALB/c sera		< 100	3000

$V_H NP^b$ and $V_H NP^a$

The idiotypic analysis in Table 4 confirmed our earlier findings that like C57BL mice, CB-20 mice produce anti-NP antibody that is positive for idiotype NP-b but negative for NP-a. BAB-14 mice resembled BALB/c in that idiotype NP-a but not NP-b is detectable in their anti-NP sera. CB-16KN mice produced NP-b positive NP-a negative antibody like C57BL and CB-20 mice.

Most mice of strain CB-8K were positive for both idiotypes but a few were a^+b^+ and a few others a^-b^+ (Table 4). This suggested that *Ig* heavy chain haplotypes were still segregating in this line in spite of the four to six generations of inbreeding. To confirm or disprove this, we mated two mice that were negative for idiotype NP-b. The resulting subline is called CB-8KN. The first nine adult members of this line were immunized with NP, and all were positive for idiotype NP-a but negative for

idiotype NP-b (Table 4). The result confirms the heterozygosity hypothesis. It suggests that the CB-8KN line has a recombinant haplotype similar or identical to the haplotype of the BAB-14 strain.

Discussion

We bred a congenic mouse strain by backcrossing a (BALB/c × C57BL/6)F_1 mouse with a BALB/c mouse, by selecting a male offspring that had the *Ig-1*^{b} allele of the C57BL parent, and by breeding this offspring with a BALB/c female. This backcrossing was continued for 16 generations, then two *Ig-1*^{a}/*I-g-1*^{b} heterozygotes were mated, and two *Ig-1*^{b}/*Ig-1*^{b} homozygotes selected from the offspring. They were bred, and constituted the nucleus of the congenic line CB-16KN which has been maintained since then by sister-brother mating.

From the offspring of the eighth backcross generation another congenic line was produced, exactly as line CB-16KN was produced from the 16th generation. This line is called CB-8K. Both of these new lines carry the C57BL alleles of the *Ig-1* locus (*C* regions of Ig H chains) and of loci closely linked to it in a predominantly BALB/c background genome. They thus closely resemble congenic strains CB-20 and BAB-14, other BALB/c-Ig-1^b strains that were originally bred by Dr. M. Potter and continued by him and by Dr. L. A. Herzenberg.

Lines CB-8K and CB-16KN were immunized with four different antigens that are known to induce antibodies of restricted heterogeneity in one of the parent strains (haptens nitro-phenyl, phenyloxazolone, and polysacharides α- (1–3) dextran and streptococcal A carbohydrate).

The most likely a priori result was that both new lines express the complete phenotype of the C57BL parent as another similarly produced congenic strain CB-20 does (Imanishi and Mäkelä 1975, Riblet et al. 1975 a, Riblet et al. 1975 b, Mäkelä et al. 1978). This strain apparently inherited an intact or nearly intact *Ig* heavy chain region from the C57BL parent.

Another reasonable a priori possibility was that strain CB-16KN would have a recombinant chromosome in the same way as another congenic BALB/c-Ig-1^b strain (BAB-14). Depending on whether the cross-over event took place before or after backcross generation eight, strain CB-8K would then have the recombinant chromosome or a complete C57BL haplotype.

Neither of the two „reasonable" expectations turned out to be true. The CB-16KN strain behaved like CB-20 but contrary to expectation the CB-8K strain, derived from the same original backcross line, had a recombinant phenotype. It expressed two markers of the C57BL parent (allotype and V_HNP^b) and four markers of the BALB/c parent (V_HDEX, V_HS117, $V_H$phOx and V_HNP^a.

Although the CB-8K line had undergone four to six generations of inbreeding at the time when most of our tests were carried out it was still heterozygous. A normal C57BL haplotype together with the recombinant haplotype was segregating in it. This was suggested by several tests and it was confirmed by mating two selected CB-8K mice who failed to express the V_HNP^b gene. All nine offspring tested also failed to express it (Table 4). The final line that was initiated from these nine offspring is called CB-8KN.

On the basis of the observed heterozygosity in the CB-8K line and because the CB-16KN line behaved like C57BL in our tests, we can conclude that the heterozygous father of backcross generation eight must have inherited a normal C57BL/6 haplotype from his father. The cross-over event must have happened either in one of his meiotic cells or in one of his heterozygous offspring who initiated the CB-8K line.

Two points of interest can be made from our data. One is the unexpectedly high recombination frequency between the allotype genes and the $V_H DEX$ gene when the C57BL and BALB/c chromosomes are segregating in a predominantly BALB/c background genome. The four BALB/c congenic strains tested in this paper represent the total of 42 backcross equivalents (opportunities for a meiotic crossing over) as follows: CB-20: 21 backcross equivalents, BAB-14: two additional backcross equivalents, CB-16KN: 17 backcross equivalents, and CB-8KN: two additional backcross equivalents. Thus the cross-over frequency is 0.048 per backcross equivalent, ten times higher than the cross-over frequency 0.004 reported for the same pair of markers (V_HDEX and allotype) in other background genomes (Riblet 1977, Weigert and Riblet 1977). The difference is on the borderline of being statistically significant ($p \sim 0.03$).

The observed difference could have been caused by different effects of the different background genomes on the recombination frequency, but this does not explain another striking fact: both the BAB-14 and the CB-8K crossing over events must have occurred at the first sister-brother generation or immediately before this generation. The former alternative is more plausible than the latter since the generations preceding the first sister-brother generation were not different from the other backcross generations. A hypothesis can be constructed which explains both the high crossing over frequency of 2/46 and the fact that both of these events occurred at (or immediately before) the first sister-brother generation.

The first sister-brother generations in the Konstanz breeding scheme differed from all other generations by including a heterozygous female. In all other generations, a heterozygous male was mated with a BALB/c female (corresponding information about the NIH breeding was not available to us). Crossing over events can only be detected in heterozygotes, and the frequency in many mouse loci is higher in females than males (Dunn and Bennett 1967). This may explain why crossing over events only took place at the sister-brother generation.

Serious objections to the above argument are that heterozygous females have certainly been included in the extensive series of Riblet and co-workers (Riblet 1977, Weigert and Riblet 1977) and that the sex difference in recombination frequencies is usually only twofold. To this we can say that the chromosomes (no. 12) carrying the genes for allotype b in the NIH and Konstanz breedings must have obtained several BALB/c alleles by generation eight of the backcrossing. If the *Ig* heavy chain region is not near one end of chromosome 12, both ends of it were probably homologous to the BALB chromosome. Homologous areas may be „sticky" to each other, and therefore increase the recombination frequency between nonhomologous regions of the same chromosome. The sex effect and the hypothetical „sticky end" effect might be synergistic. This has not been systematically studied, but the data of Riblet and Weigert (Weigert and Riblet 1977, Riblet 1977) are compatible with the „sticky end" hypothesis. They found eight cross-over chromosomes per 2460 chromosomes

tested (0.32 per cent) when the cromosomes had no known homologies. The number was 5 per 882 (0.57%) when the chromosomes probably had homologies (the cross was (BAB-14 × C. AL-9)F_1 × C.AL-9).

References

Berek, C., Taylor, B. A., and Eichmann, K.: Genetics of the idiotype of BALB/c myeloma S117: multiple chromosomal loci for V_H genes encoding specificity for Group A streptococcal carbohydrate. *J. Exp. Med. 144*: 1164–1174, 1976

Blomberg, B., Geckler, W. R., and Weigert, M.: Genetics of the antibody response to dextran in mice. *Science 117*: 178–180, 1972

Dunn, L. C. and Bennett, D.: Sex differences in recombination of linked genes in animals. *Genet. Res. 9*: 211–220, 1967

Eichmann, K.: Idiotypic identity of antibodies to streptococcal carbohydrate in inbred mice. *Eur. J. Immunol. 2*: 301–307, 1972

Eichmann, K.: Genetic control of antibody specificty in the mouse. *Immunogenetics 2*: 491–506, 1975

Eichmann, K. and Kindt, T. J.: The inheritance of individual antigenic specificities of rabbit antibodies to streptococcal carbohydrates. *J. Exp. Med. 134*: 532–552, 1971

Fathman, C. G., Pisetsky, D. S., and Sachs, D. H.: Genetic control of the immune response to nuclease. V. Genetic linkage and strain distribution of anti-nuclease idiotypes. *J. Exp. Med. 145*: 569–577, 1977

Imanishi, T. and Mäkelä, O.: Inheritance of antibody specificity. II. Anti-(4-hydroxy-5-bromo-3-nitrophenyl) acetyl in the mouse. *J. Exp. Med. 141*: 840–854, 1975

Karjalainen, K.: Two major idiotypes in mouse anti-NP antibodies are controlled by „allelic" genes. *Eur. J. Immunol.*, in press, 1979

Karjalainen, K. and Mäkelä, O.: A mendelian idiotype is demonstrable in the heteroclitic anti-NP antibodies of the mouse. *Eur. J. Immunol. 8*: 105–112, 1978

Leon, M. A., Young, N. M., and McIntire, K. R.: Immunochemical studies of the reaction between a mouse myeloma macroglobulin and dextran. *Biochemistry 9*: 1023–1030, 1970

Lieberman, R., Potter, M., Humphrey, W. Jr., and Chien, C. C.: Idiotypes of inulinbinding antibodies and myeloma proteins controlled by genes linked to the allotype locus of the mouse. *J. Immunol. 117*: 2105–2111, 1976

Mäkelä, O., Kaartinen, M., Pelkonen, J. L. T., and Karjalainen, K.: Inheritance of antibody specificity. V. Anti-2-Phenyloxazolone in the Mouse. *J. Exp. Med. 148*: 1644–1660, 1978

Riblet, R. J.: The organization of antibody genes, *ICN-UCLA Symposia on Molecular and Cellular Biology 6*: 83–89, 1977

Riblet, R., Blomberg, B., Weigert, M., Lieberman, R., Taylor, B. A., and Potter, M.: Genetics of mouse antibodies. I. Linkage of the dextran response locus, V_H-DEX, to allotype. *Eur. J. Immunol. 5*: 775–777, 1975a

Riblet, R., Weigert, M., and Mäkelä, O.: Genetics of mouse antibodies. II. Recombination between V_H genes and allotype. *Eur. J. Immunol. 5*: 778–781, 1975b

Weigert, M. and Riblet, R.: Genetic control of antibody variable regions. *Cold Spring Harbor Symp. Quant. Biol. 41*: 837–846, 1977

Weiler, I. J., Weiler, E., Sprenger, R., and Cosenza, H.: Idiotype suppression by maternal influence. *Eur. J. Immunol. 7*: 591–597, 1977

Reiceived August 21, 1979