# INHERITANCE OF ANTIBODY SPECIFICITY

## II. Anti-(4-Hydroxy-5-Bromo-3-Nitrophenyl)Acetyl in the Mouse*

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Variable (V)[1] genes code for the variable polypeptide sequence of immunoglobulin molecules. Available evidence indicates that there are three pools of V genes, one shared by all kappa chain subtypes, another by lambda chain subtypes, and the third by all heavy chain classes and subclasses. In the case of kappa and lambda V genes the evidence is mainly sequence data of myeloma proteins whereas several additional pieces of evidence suggest that all heavy chains share a common pool of variable polypeptide sequences. The structural genes for these sequences are called $V_H$ genes.

$V_H$ genes of the rabbit and the mouse have been defined on Mendelian terms. In the rabbit the *a*-locus allotypes, $a_1$, $a_2$, and $a_3$, have helped in gaining the important information that V and constant (C) genes of the heavy chain remain close but not absolute linkage (1). Meiotic recombinations occur at a frequency of approximately 0.3% (2, 3).

Different Mendelian markers of $V_H$ genes have been observed in the mouse. Contrary to $a_1$, $a_2$, and $a_3$ allotype markers of the rabbit, each of the mouse $V_H$-gene markers is present in only a small proportion of immunoglobulin molecules sharing an antibody specificity. Both this and the mapping data suggest that each of the mouse markers represents a small proportion of all V genes.

At least six Mendelian $V_H$-gene markers have been published: the gene controlling the idiotype found in antiarsonate antibodies of A/J and AL/N mice (4), the gene controlling the idiotype in the antistreptococcal A carbohydrate antibody in A/J mice (5), the gene controlling the anti-alpha-1-3-dextran response in BALB/c mice (6), the gene controlling the T15 idiotype found in the anti-phosphorylcholine antibody in BALB/c mice (7), the gene controlling the fine specificity of anti-(4-hydroxy-3-nitrophenyl)acetyl antibodies in C57BL/6 mice (8), and the gene controlling the antiphosphorylcholine antibody with the S107 idiotype in the BALB/c mice (9). The six markers are based on three different

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[1] *Abbreviations used in this paper:* aminocap, *N*, ε-amino-*n*-caproic acid; BSA, bovine serum albumin; CG, chicken globulin; DIP, (4-hydroxy-3-5-diiodophenyl)acetyl; FSI, fine specificity index (see Results section); HPI, haptenated phage inactivation; HPII, haptenated phage inactivation inhibition; $I_{50}$, concentration of hapten causing 50% inhibition of a serological reaction; $IgC_H$, immunoglobulin heavy chain constant region; 2-ME, 2-mercaptoethanol; NBrP, (4-hydroxy-5-bromo-3-nitrophenyl)acetyl; NClP, (4-hydroxy-5-cloro-3-nitrophenyl)acetyl; NIP, (4-hydroxy-5-iodo-3-nitrophenyl)acetyl; NNP, (4-hydroxy-3,5-dinitrophenyl)acetyl; NP, (4-hydroxy-3-nitrophenyl)acetyl; V, variable; $V_H$ gene, coding for the heavy chain V region.

technical principles: (*a*) most mice of the same genotype share an idiotype or an isoelectric-focusing pattern in specific antibody, e.g., antistreptococcal A carbohydrate (5); (*b*) mice of the same genotype are either high or low responders to an antigenic determinant, e.g., alpha-1-3-dextran (6); and (*c*) mice of the same genotype have a characteristic fine specificity in their antibody to an antigenic determinant (8). In this paper we report a V gene that manifests itself by causing a high response to hapten (4-hydroxy-5-bromo-3-nitrophenyl)acetyl (NBrP) and a characteristic fine specificity in this antibody.

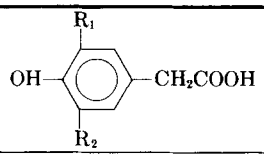
## Materials and Methods

*Mice.* Most of our mouse strains were obtained from the Jackson Laboratories, Bar Harbor, Maine. CBA, C3H, BALB/c, C57BL/6, DBA/2N, and IAH strains were from our colony. Strain C57BL/Ka and CB20 were kindly given us by Dr. Michael Potter (National Cancer Institute, NIH, Bethesda, Md.).

*Haptens and Their Conjugates.* The haptens used are shown in Table I. Most of them were prepared according to the method of Brownstone et al. (10). NBrP and its derivatives and (4-hydroxy-5-iodo-3-nitrophenyl)acetyl (NIP) azide were prepared as previously described (11). (4-hydroxy-5-cloro-3-nitrophenyl)acetyl (NClP) was prepared by adding 2 g of (4-hydroxy-3-nitrophenyl)acetyl (NP) into 25 ml of glacial acetic acid in a 1 liter stoppered flask. The flask was filled with chlorine under a hood, stoppered quickly, and shaken overnight at room temperature under the hood. The next day it was refilled with chlorine and incubated over another night. The resulting yellow crystals were washed with acetic acid and dried. When dissolved they had an extinction maximum at $\lambda$ of 430, and the coefficient of extinction was 4,800. This extinction was pH dependent, and the pH midpoint for the conversion from colored to noncolored was 5.4. This was indistinguishable from the corresponding values for NIP and NBrP but different from the NP value (pH 7.2). The method for preparing further derivatives of NClP was analogous to the method for preparing NBrP derivatives.

TABLE I

*Haptens Used in This Study*




| Compound | $R_1$ | $R_2$ |
|---|---|---|
| NBrP | Br | $NO_2$ |
| NIP | I | $NO_2$ |
| NClP | Cl | $NO_2$ |
| NNP | $NO_2$ | $NO_2$ |
| NP | H | $NO_2$ |
| DIP | I | I |

*Immunization of Mice.* The immunogens were NBrP coupled to chicken globulin (CG) or bovine serum albumin (BSA). CG was obtained from blood serum by 45% ammonium sulphate saturation. BSA (Cohn fraction V) was obtained from Armour and Company, Ltd., Eastbourne, England. $NBrP_{14}CG$ contained 14 mol of NBrP/150.000 g of CG, and $NBrP_{14}BSA$ contained 14 mol of NBrP/mol of BSA. Alum-precipitated $NBrP_{14}CG$ (100 $\mu$g/mouse/injection) was injected intraperitoneally with (first injection) or without (second injection) $10^9$ *Bordetella pertussis* bacteria as adjuvant. $NBrP_{14}BSA$ (100 $\mu$g) was injected subcutaneously (four sites) in complete Freund's adjuvant. The second injection was administered 40 days after the first. The serum antibodies from mice were obtained by bleeding 12 days after the second injection if not otherwise mentioned.

*Antibody Assays.* The amount of antibody was measured by inactivation of haptenated (NBrP) bacteriophage (HPI test) which has been described earlier (11).

*Affinity Assays.* Six free haptens in the form of *N*, ϵ-amino-*n*-caproic acid (aminocap) derivatives were used: NBrP aminocap, NIP aminocap, NClP aminocap, (4-hydroxy-3.5-dinitrophenyl)acetyl (NNP) aminocap, NP aminocap, and (4-hydroxy-3-5-diiodophenyl)acetyl (DIP) aminocap. Affinity of the antibodies was estimated by inhibiting the HPI reaction with varying concentrations of the above haptens.

*Antiallotype Sera.* Anti-Ig-$1^b$ was obtained by immunizing BALB/c mice with *B. pertussis* bacteria coated with C57BL/6 antibodies according to Dresser and Wortis (12). Anti-Ig-$1^a$ was prepared by immunizing C57BL/6 mice with *B. pertussis* bacteria coated with BALB/c antibodies. The typing was done by the double-diffusion method.

## Results

*Anti-NBrP Antibodies in Mouse Sera; Affinity and Fine Specificity.* The general method for studying fine specificity was hapten inhibition of NBrP-phage inactivation (HPII). Five related haptens including the immunogenic NBrP were used for the inhibition. The relationship of hapten concentration to HPI inhibition is illustrated in Fig. 1. $I_{50}$ values were interpolated from the crossing of the inhibition curves and the 50% horizontal line. Reasonably constant and accurate $I_{50}$ values could be obtained by using half-log steps in the concentration series.

The main characteristics of anti-NBrP produced by the BALB/c and C57BL/6 mice emerge from Fig. 1 (individual mice) and Table II (mean $I_{50}$ values). BALB/c antibodies had the highest affinity for the immunogenic NBrP but both NIP and NClP followed closely thereafter. Affinity for NNP was lower and for NP lower still. C57BL/6 antibodies, on the other hand, had a high affinity for NBrP, NIP, and NNP while their affinity for both NP and NClP was much lower.

Maturation of the response was seen in both strains of mice. It manifested itself

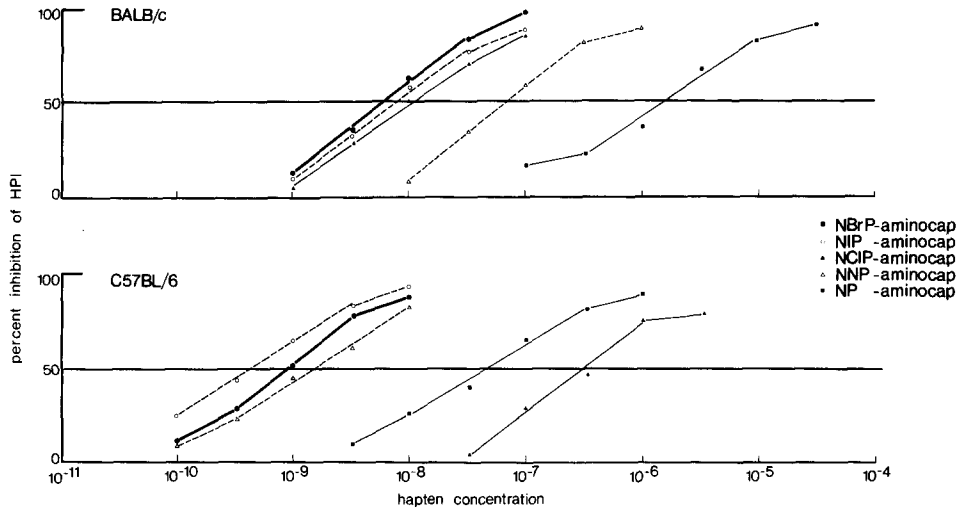



FIG. 1. Inhibition of anti-NBrP by NBrP-aminocap and related haptens. Antibodies were obtained from a BALB/c or a C57BL/6 mouse 12 days after the second injection of NBrP-CG. A hapten concentration was said to cause 50% inhibition of NBrP-T4 inactivation (HPI) if it increased the number of phage survivors to the survival value of twice diluted antibody, 80% inhibition corresponded to the phage survivor count of fivefold diluted antibody, etc.

TABLE II

*Affinity of Anti-NBrP for Various Haptens at Different Stages of Immunization*

| Strain | No. of mice | Time after immunization (days) | $I_{50}$* values for: | | | | |
|---|---|---|---|---|---|---|---|
| | | | NBrP-aminocap | NIP-aminocap | NNP-aminocap | NP-aminocap | NClP-aminocap |
| | | *days* | | | | | |
| BALB/c | 7 | 14 | 130 | 110 | 610 | 21,000 | 87 |
| | 8 | 17 | 21 | 45 | 250 | 4,400 | 18 |
| | 8 | 29 | 6 | 9.7 | 69 | 1,600 | 16 |
| | 21 | 12 sec‡ | 5.8 | 9.8 | 67 | 1,600 | 15 |
| C57BL/6 | 7 | 14 | 63 | 46 | 77 | 2,100 | 1,200 |
| | 7 | 17 | 20 | 11 | 34 | 620 | 320 |
| | 7 | 29 | 3.4 | 2.2 | 5.9 | 100 | 100 |
| | 18 | 12 sec | 2.4 | 1.4 | 4.5 | 60 | 140 |

* As primary response sera were tested in the presence of 2-ME the data are representative of 7S antibodies only. $I_{50}$ values are geometric means of the nanomolar hapten concentrations causing 50% inhibition of NBrP-T4 phage inactivation (8).

‡ 12 days after the secondary immunization (sec).

in two ways: (*a*) $I_{50}$ values for all haptens decreased, and (*b*) relative affinities for haptens other than the immunogenic one decreased. By this criterion, antibodies grew more "specific" for the immunogen. In spite of this maturation the strain characteristics remained clearly visible at all stages.

CG was the carrier molecule for NBrP in most of these experiments. We did a limited experiment using NBrP-BSA as the immunogen. Affinity of the anti-NBrP produced by BALB/c and C57BL/6 was lower than when NBrP-CG was used, but the relative affinities were again the same as those for the anti-$NBrP_{14}CG$ response (Table III).

Other inbred strains of mice were tested for the fine specificity of their anti-NBrP. The results indicate that mouse strains can be divided into at least three categories on the basis of their anti-NBrP (Table IV).

Category 1 is characterized by low $I_{50}$ values (high relative affinity) for NIP and NNP, relatively high affinity for DIP and NP, but low affinity for NClP. All five tested allotype Ig-$1^b$ strains belonged to this group.

Category 2 is characterized by very low affinity for DIP and NP, and by high affinity for NClP (higher than for NNP). Most tested strains (BALB/c, MA/J, C57L, ST/bJ, IAH, DBA/2N, A/J, and RF/J) belonged to this category, including several allotype Ig-$1^a$ strains.

Category 3 had only two strains, CBA, and C3H of allotype Ig-$1^a$ (and perhaps the only tested allotype Ig-$1^d$ strain AKR). The anti-NBrP of this category differed from that of category 2 by its high affinity for NBrP, DIP, NIP, and NNP. It differed from category 1 by having lower affinity for NP than for NClP. The low number of AKR mice makes the classification of this strain unreliable.

*Inheritance of the Strain Characteristics.* We wanted to present the data of individual mice in a two-dimensional form, and for this purpose the fine

TABLE III

*Amount and Affinity of Anti-NBrP Antibodies in the Secondary Response to NBrP-BSA*

| Strain | No. of mice | ME-resistent titer (log)* | $I_{50}$‡ values for: | | | | |
|---|---|---|---|---|---|---|---|
| | | | NBrP-aminocap | NIP-aminocap | NNP-aminocap | NP-aminocap | NClP-aminocap |
| BALB/c | 10 | 6.4 ± 0.09 | 18 | 26 | 160 | 5,000 | 13 |
| C57BL/6 | 7 | 5.2 ± 0.14 | 8.3 | 2.5 | 12 | 105 | 180 |

* Animals were bled 12 days after the second injection. NBrP-T4 phage was used for HPI and HPII tests. All the tests were performed using 2-ME to inactivate IgM antibodies. Titers are given as log mean ± standard error.

‡ Values are geometric means of nanomolar hapten concentrations causing 50% reduction of the phage inactivation.

TABLE IV

*Affinity of Anti-NBrP Antibody for NBrP and Related Haptens in Different Inbred Strains of Mice*

| Strain | *H-2* | $IgC_H$ allotype | No. of mice | $I_{50}$* values for: | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | NBrP-aminocap | NIP-aminocap | NNP-aminocap | NP-aminocap | NClP-aminocap | DIP-aminocap |
| CBA | *k* | Ig-1$^a$ | 10 | 2.9 | 1.4 | 8.7 | 530 | 65 | 50 |
| C3H | *k* | Ig-1$^a$ | 12 | 2.9 | 1.4 | 8.3 | 340 | 180 | 27 |
| BALB/c | *d* | Ig-1$^a$ | 21 | 5.8 | 9.8 | 67 | 1,600 | 15 | 10,100 |
| MA/J | *k* | " | 4 | 6.9 | 8.7 | 49 | 2,300 | 9.5 | 3,900 |
| C57L | *b* | " | 6 | 4.6 | 7.6 | 51 | 1,400 | 10 | 800 |
| ST/bJ | *k* | " | 6 | 5.3 | 7.7 | 27 | 1,700 | 7.5 | 2,800 |
| IAH | | " | 8 | 5.8 | 13 | 74 | 1,800 | 13 | 2,400 |
| C57BL/6 | *b* | Ig-1$^b$ | 18 | 2.4 | 1.4 | 4.5 | 60 | 140 | 180 |
| C57BL/Ka | *d* | " | 14 | 2.3 | 2.2 | 5.6 | 68 | 200 | ND |
| C57BL/Ks | *d* | " | 5 | 1.8 | 0.91 | 5.8 | 110 | 110 | ND |
| LP/J | *b* | " | 14 | 1.6 | 0.98 | 5.1 | 93 | 120 | ND |
| SJL/J | *s* | " | 10 | 2.0 | 1.4 | 6.7 | 270 | 340 | ND |
| RF/J | *k* | Ig-1$^c$ | 6 | 4.0 | 2.7 | 23 | 600 | 14 | 880 |
| DBA/2N | *d* | Ig-1$^c$ | 11 | 4.7 | 7.9 | 33 | 1,200 | 8.8 | 9,400 |
| AKR | *k* | Ig-1$^a$ | 5 | 2.0 | 1.1 | 12 | 760 | 46 | ND |
| A/J | *a* | Ig-1$^a$ | 6 | 2.9 | 5.9 | 66 | 2,100 | 61 | ND |
| CE/J | *k* | Ig-1$^f$ | 10 | 3.6 | 4.4 | 66 | 1,900 | 19 | ND |

* All the animals were bled 12 days after secondary immunization and were tested with the HPII method, with NBrP-T4 phage. All values are geometric means of the nanomolar hapten concentrations causing 50% reduction of the phage inactivation.

specificity index (FSI) was calculated for each mouse. For the index we selected the most informative haptens NNP, NP, and NClP. We found a high positive correlation between the affinities for NP and for NNP. High affinity for NClP had a negative correlation to high affinity for NNP and for NP. This suggested an index where the $I_{50}$ value for NClP was divided by the mean of corresponding NNP and NP values. We found, however, that the scatter between individual

mice was reduced by using the mean of NClP and NBrP values as the denominator, and adopted this index:

$$FSI = \sqrt{\frac{I_{50}(NBrP) \times I_{50}(NClP)}{I_{50}(NNP) \times I_{50}(NP).}}$$

When the indices of individual animals were plotted (Fig. 2) the three groups of strains could again be distinguished. There was no overlap between mice of categories 1 and 2 while mice of category 3 were located between them.

The FSIs of the anti-NBrP antibodies of 21 BALB/c and 18 C57BL/6 mice were distributed as two nonoverlapping populations (Fig. 2). The gap between these two distributions made a satisfactory basis for testing crosses between these two strains. The frequency distribution of 24 $F_1$ hybrid mice tested suggested complete dominance of the BALB/c trait (Fig. 3). The mean of these $F_1$ mice was 0.033 compared to the mean of 0.026 in the BALB/c and 0.95 in C57BL/6.

Backcrossing into the recessive C57BL/6 strain produced 52 offspring. The distribution of the specificity indices was bimodal with distinct peaks coinciding either with BALB/c or C57BL/6 peaks. Since there was a correlation between the allotype and the specificity index, Ig-$1^{ab}$ heterozygotes and Ig-$^{bb}$ homozygotes were separated in Fig. 3. The correlation turned out to be absolute. All Ig-$1^{ab}$ mice had an index of $<0.4$ while all Ig-$1^{bb}$ homozygotes had an index of $>0.4$. These results indicate that the fine specificity of anti-NBrP antibodies is controlled by one (or more) allotype-linked gene(s).

*Fine Specificity Characteristics in Congenic and Recombinant Inbred Strains of Mice.* In an attempt to further study the roles of allotype-linked and other genes in the fine specificity of anti-NBrP antibodies, we tested CB20 mice that have the Ig heavy chain constant region ($IgC_H$) genes of C57BL/Ka in a BALB/c background genome (7), and the Bailey recombinant inbred strains that have different combinations of C57BL/6 and BALB/c genes (13). Finally we studied three congenic strains carrying different *H-2* alleles in a C57BL/10Sn background.

The specificity of the secondary response anti-NBrP antibodies in these strains was exclusively determined by a gene(s) linked to the *Ig-1* locus (Fig. 4). CB20 antibody was indistinguishable from the anti-NBrP of the $IgC_H$ donor and very different from the BALB/c antibody. The Bailey recombinant strains, C × BD, C × BE, C × BH, C × BI, and C × BK (Ig-$1^b$) produced anti-NBrP that closely resembled C57BL/6 anti-NBrP while only C × BJ and C × BG strains (Ig-$1^a$) had low specificity indices characteristic of BALB/c.

Our data do not suggest that *H-2*-linked genes have a role in determining the specificity of the anti-NBrP antibodies. Mice with C57BL/10Sn background were indistinguishable regardless of whether their *H-2* allele was *a*, *k*, or *d* (Fig. 4).

Table V presents the geometric mean of $I_{50}$ values for each hapten in mice of the tested genotypes. It shows that any one of the haptens NNP, NP, or NClP could have been used alone for the demonstration of the anti-NBrP genetic marker.

*Antibody Concentrations.* BALB/c mice had higher anti-NBrP titers than

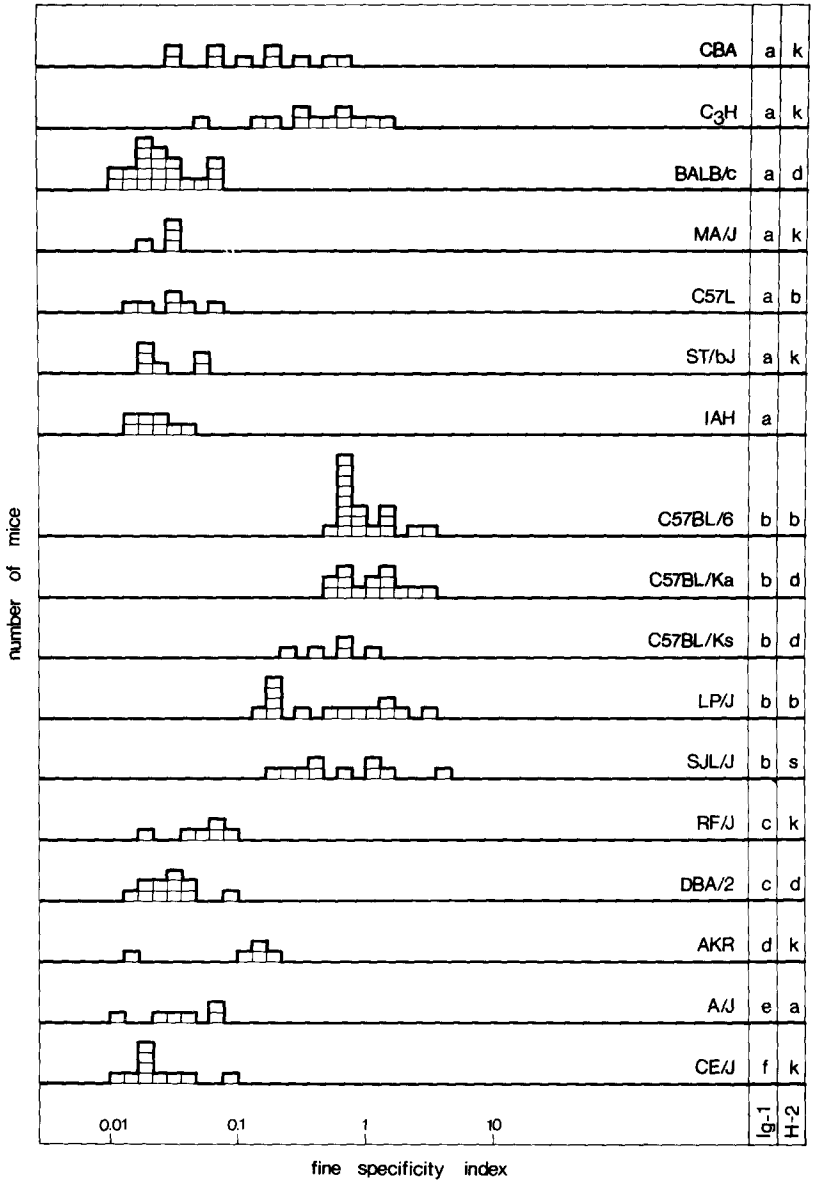



FIG. 2. Frequency distribution of FSIs (see text) in anti-NBrP of different mouse strains. Each mouse is represented by a box whose horizontal location indicates the FSI. Antibodies were obtained 12 days after the second injection of NBrP-CG.

C57BL/6 mice at the different stages of immunization (Table VI). The high secondary response of the BALB/c was associated with fine specificity in all but one type of mice tested. It was dominant over the C57BL/6 allele and segregated in the backcross generation in linkage with the allotype (Fig. 5 and Table VI). In the congenic and recombinant strains high response was associated with Ig-$1^a$ allotype and low response with Ig-$1^b$ allotype in 10 cases, but one strain behaved

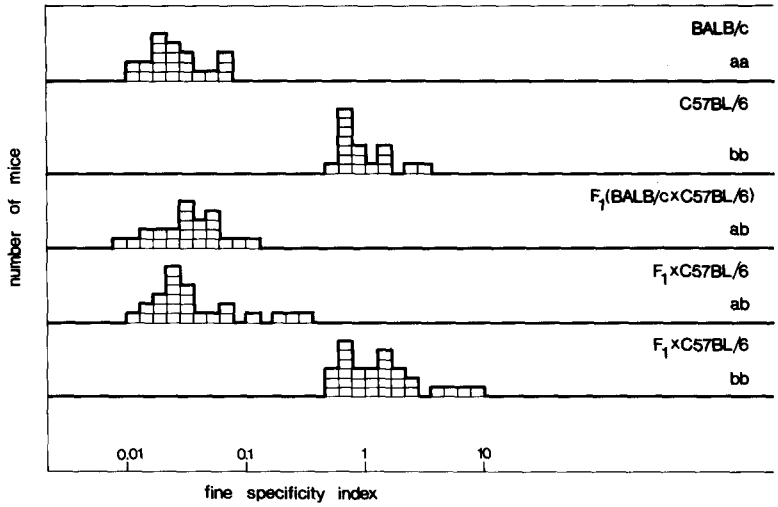



FIG. 3. Inheritance of the fine specificity. The backcross generation was divided into those heterozygous or homozygous for the heavy chain allotype. For explanations see Fig. 2.

differently (Table VII). The B10-Br mouse strain that carries the Ig-1$^b$ allotype and had the corresponding high FSI was a high responder.

All allotype Ig-1$^a$ strains had a high response as had the BALB/c, and all allotype Ig-1$^b$ strains except B10-Br a low response. Mice of the non-a and non-b allotype, RF/J, DBA/2N, A/J, and CE/J had a high response and a low FSI. An exception was AKR but here the number of mice was low (Table VII).

## Discussion

We found that mice of different strains produced qualitatively different antibodies to hapten NBrP when they were immunized by NBrP conjugates of BSA or CG. The differences were demonstrated by determining the relative affinities of the antibodies to related haptens NIP, NNP, NP, NClP, and DIP. On the basis of the fine specificity patterns of their anti-NBrP, mouse strains could be divided into three categories which correlated strongly but not absolutely with the Ig heavy chain allotype. All five strains of allotype b belonged to one category. All allotype a strains, except CBA and C3H, and all non-a and non-b strains tested belonged to the second category. CBA and C3H strains (the third category) were intermediate between the first and the second category. The AKR strain could not be reliably classified, only five mice were tested.

Associated with the fine specificity polymorphism was a polymorphism in the magnitude of the anti-NBrP response. Mouse strains of the first fine specificity category produced lower antibody titers than mice of the second and the third category. The difference in anti-NBrP titers between C57BL/6 and BALB/c mice persisted throughout the primary and the secondary responses. Apart from the data in Tables III and VI, we found a similar difference at day 7 of the primary response (log means, 2.9 for C57BL/6 and 3.8 for BALB/c). This was interesting since at that time all antibody was 2-mercaptoethanol (2-ME) sensitive and poorly

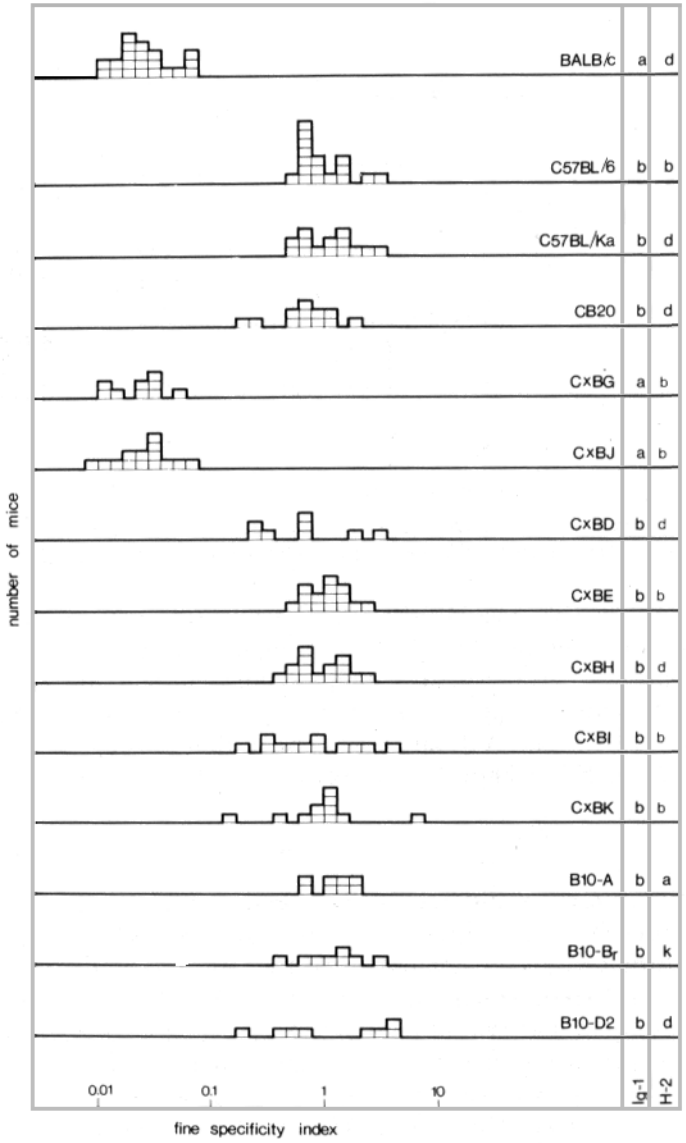



FIG. 4. Frequency distribution of FSIs among mice of different genotypes. For explanations see Fig. 2.

inhibitable by free hapten; thus it probably was IgM. From this we conclude that allotype-linked immune responsiveness genes control the IgM response too. This is clearly not the case with the *H*-2-linked *Ir*-genes (14).

$F_1$ hybrid animals between a category 1 strain (C57BL/6) and a category 2 strain (BALB/c) were indistinguishable from BALB/c mice. Hybrids between the $F_1$ and the recessive C57BL/6 could be divided into bb allotype homozygotes and ab heterozygotes. Homozygotes were indistinguishable from C57BL/6 parents

TABLE V

*An Allotype-Linked Gene Controls the Fine Specificity of Anti-NBrP Antibodies*

| Strain | $IgC_H$ allotype (Ig-1) | No. of mice | $I_{50}$* values for: | | | | |
|---|---|---|---|---|---|---|---|
| | | | NBrP-aminocap | NIP-aminocap | NNP-aminocap | NP-aminocap | NClP-aminocap |
| Parental strains | | | | | | | |
| BALB/c | aa | 21 | 5.8 | 9.8 | 67 | 1,600 | 15 |
| C57BL/6 | bb | 18 | 2.4 | 1.4 | 4.5 | 60 | 140 |
| C57BL/Ka | bb | 14 | 2.3 | 1.8 | 5.6 | 68 | 200 |
| $F_1$ progeny | | | | | | | |
| BALB/c × C57BL/6 | ab | 23 | 6.7 | 7.9 | 49 | 910 | 10 |
| Backcross progeny | | | | | | | |
| $F_1$ × C57BL/6 | ab | 23 | 6.2 | 9.2 | 51 | 1,400 | 18 |
| $F_1$ × C57BL/6 | bb | 29 | 2.7 | 2.1 | 3.4 | 84 | 180 |
| Congenic strain (Ig) | | | | | | | |
| CB20 | bb | 12 | 2.5 | 1.2 | 4.3 | 120 | 107 |
| Bailey Recombinant inbred strains | | | | | | | |
| C × BG | aa | 9 | 4.2 | 5.5 | 32 | 1,900 | 8 |
| C × BJ | aa | 14 | 9.2 | 16 | 69 | 2,000 | 10 |
| C × BD | bb | 8 | 2.3 | 2.2 | 10 | 170 | 350 |
| C × BE | bb | 15 | 1.7 | 1.4 | 4.1 | 69 | 180 |
| C × BH | bb | 15 | 1.9 | 0.9 | 5.8 | 96 | 230 |
| C × BI | bb | 12 | 2.9 | 1.3 | 13 | 120 | 310 |
| C × BK | bb | 11 | 3.6 | 1.7 | 4.9 | 82 | 107 |
| Congenic strains (H-2) | | | | | | | |
| B10-D2 *H-2d* | bb | 8 | 1.5 | 0.6 | 2.2 | 47 | 100 |
| B10-A *H-2a* | bb | 8 | 2.6 | 1.2 | 5.5 | 110 | 315 |
| B10-Br *H-2k* | bb | 8 | 2.1 | 0.7 | 3.6 | 75 | 140 |

* Animals were bled 12 days after the second injection. NBrP-T4 phage was used for HPII. Values are geometric means of nanomolar hapten concentrations causing 50% reduction of the phage inactivation.

and heterozygotes indistinguishable from the BALB/c mice with regard to the fine specificity and the magnitude of the anti-NBrP response.

These data suggested that both observed polymorphisms were controlled by an allotype-linked gene(s). This was confirmed by immunization experiments of congenic and recombinant inbred strains of mice. 11 such strains were immunized. Eight of them contained C57BL and BALB/c genes in various combinations. Whenever these mice carried the Ig-$1^a$ allotype of the BALB/c they were high responders to NBrP, and the fine specificity resembled BALB/c. All strains carrying the C57BL allotype produced anti-NBrP with C57BL type fine specificity regardless of the other genes. All these strains but one (B10-Br) were low responders. We cannot explain this exception.

The data indicate that the amount and fine specificity of the anti-NBrP antibodies of the BALB/c mice mark a $V_H$ gene. This V gene is dominant over the C57BL/6 allele. Data of Table II and Fig. 2 suggest that CBA and C3H mice may

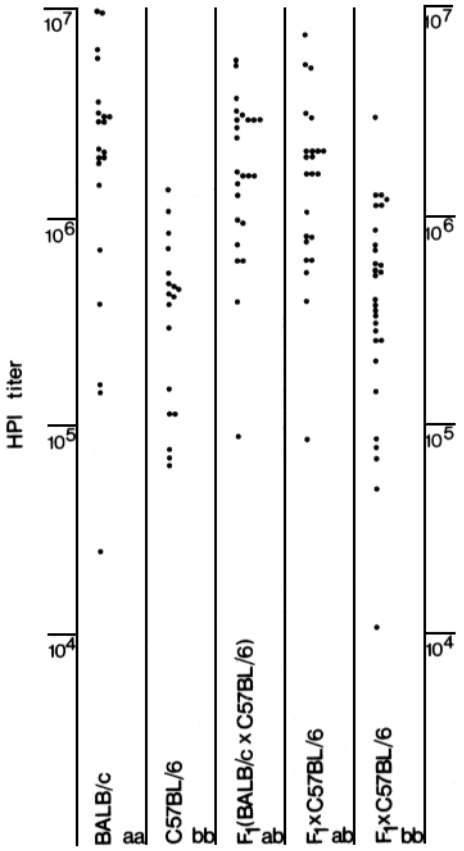



FIG. 5. Mean anti-NBrP titers of mice with different genotypes. Sera were obtained 12 days after the second injection of NBrP-CG.

have a third allele of the same locus. This allele would be recessive to the BALB/c allele since the $F_1$ mice were like BALB/c (unpublished experiments) but a proper study was not conducted since both strains belong to allotype a and a marker for the *Ig-1* locus was not available.

Anti-NBrP antibodies of the BALB/c mice had a lower affinity for NBrP than those of the C57BL/6 mice. This "low" affinity was dominant over the "high" affinity of the C57BL/6 antibody in $F_1$, and it was linked to the allotype in the backcross mice (Table V). A corresponding affinity difference between BALB/c and C57BL/6 could be demonstrated by using radioactive antigen-binding test (unpublished data) and in the primary response to NBrP-CG. It could be demonstrated in anti-NBrP antibodies induced by NBrP-BSA (Table III). Therefore the low affinity of the BALB/c antiNBrP antibodies seems to be exclusively controlled by the $V_H$ gene that causes a high response to NBrP and a low FSI. The dominance of the low affinity is difficult to explain as is its concordance with high total response. As the BALB/c allele was dominant in both fine specificity and the quantity of the response, its product should have had a higher affinity for the immunogen than the product of the recessive allele. The only possible explanation we can offer at this stage is that whenever the BALB/c allele was present in a hybrid mouse it was expressed in much more numerous B lymphocytes than the C57BL/6 allele.

TABLE VI
*Control by an Allotype-Linked Gene of the Concentration of Anti-NBrP Antibodies*

| | No. of mice | *H-2* | $IgC_H$ allotype (Ig-1) | Days after immunization | HPI titer* |
|---|---|---|---|---|---|
| Parental strains | | | | | |
| BALB/c | 8 | *d* | aa | 17 | H 4.20 ± 0.09‡ |
| | 8 | *d* | aa | 29 | H 5.32 ± 0.09 |
| | 21 | *d* | aa | 12 sec§ | H 6.20 ± 0.13 |
| C57BL/6 | 7 | b | bb | 17 | L 3.27 ± 0.24 |
| | 7 | *b* | bb | 29 | L 4.00 ± 0.29 |
| | 18 | *b* | bb | 12 sec | L 5.53 ± 0.10 |
| $F_1$ progeny | | | | | |
| BALB/c × C57BL/6 | 23 | *db* | ab | 12 sec | H 6.18 ± 0.09 |
| Backcross progeny | | | | | |
| $F_1$ × C57BL/6 | 23 | | ab | 12 sec | H 6.14 ± 0.09 |
| $F_1$ × C57BL/6 | 29 | | bb | 12 sec | L 5.55 ± 0.10 |

* Titers marked with the letter H are high and differ significantly ($P < 0.005$) from the titers of the corresponding group, marked with the letter L.
‡ Log means ± standard error.
§ sec, secondary immunization.

The NBrP gene is one of the several known V genes (4–9) linked to the heavy chain allotypes. As there are no published reports on inherited idiotypes or fine specificity characteristics unlinked to the $IgC_H$ complex gene (Sher and Cohn [9] reported that expression of S107 idiotype was regulated by two genes, one of them allotype linked and the other perhaps *H-2* linked) the data begin to suggest that the $V_H$ gene is more important for the antibody than the $V_L$. This was a priori unexpected since the variability of the light chain V regions is great and it correlates with antibody specificity as does the $V_H$ polypeptide sequence (15, 16).

As none of the 53 backcross mice tested recombined allotype Ig-$1^a$ and high FSI, or allotype Ig-$1^b$ and low FSI the frequency of crossing-overs between the Ig-1 and the *NBrP* loci is likely to be less than 5%. This is also suggested by a linkage disequilibrium between the two loci. All five allotype b strains had a high FSI while five out of seven studied allotype a strains had a low FSI. One crossing-over event between the two loci seems to be on the record however: the BAB/14 strain that is a homozygotized hybrid between the C57BL/Ka and BALB/c strains seems to combine the allotype from C57BL/Ka and the NBrP gene from the BALB/c (Mäkelä et al., unpublished observation). It also has inherited the alpha-1-3-dextran marker from the BALB/c (6, 17).

CBA and C3H were clearly different from the other five allotype *a* strains studied. Both these closely related strains were likewise different from other allotype a strains with regard to the unrelated V-gene marker of Lieberman et al. (7) and of Blomberg et al (6). This suggests that the $IgC_H$ chromosomal region of a common ancestor of CBA and C3H may have received a V-gene portion from a non-a mouse through recombination.

Since NBrP and NP are related haptens the possibility must be considered that the BALB/c gene described in this paper and the C57BL/6 gene of a pre-

TABLE VII

*Anti-NBrP Antibody Concentration in the Sera of Different Strains of Mice*

| Strain | H-2 | $IgC_H$ allotype | No. of mice | HPI titer* |
|---|---|---|---|---|
| CBA | *k* | Ig-1$^a$ | 10 | 6.49 ± 0.10 |
| C3H | *k* | Ig-1$^a$ | 12 | 5.80 ± 0.19 |
| BALB/c | *d* | Ig-1$^a$ | 21 | 6.20 ± 0.13 |
| MA/J | *k* | " | 4 | 5.96 ± 0.12 |
| C57L | *b* | " | 6 | 6.35 ± 0.09 |
| ST/bJ | *k* | " | 6 | 6.45 ± 0.22 |
| IAH | | " | 8 | 5.94 ± 0.26 |
| C × BG | *b* | " | 9 | 6.20 ± 0.17 |
| C × BJ | *b* | " | 14 | 5.94 ± 0.16 |
| C57BL/6 | *b* | Ig-1$^b$ | 18 | 5.53 ± 0.10 |
| C57BL/Ka | *d* | " | 14 | 5.70 ± 0.17 |
| C57BL/Ks | *d* | " | 5 | 5.05 ± 0.33 |
| LP/J | *b* | " | 14 | 5.23 ± 0.11 |
| SJL/J | *s* | " | 10 | 5.43 ± 0.21 |
| CB20 | *d* | " | 12 | 5.55 ± 0.13 |
| C × BD | *d* | " | 8 | 5.74 ± 0.12 |
| C × BE | *b* | " | 15 | 5.74 ± 0.11 |
| C × BH | *d* | " | 15 | 5.40 ± 0.16 |
| C × BI | *b* | " | 12 | 5.21 ± 0.13 |
| C × BK | *b* | " | 11 | 5.74 ± 0.12 |
| B10-D2 | *d* | " | 8 | 5.03 ± 0.12 |
| B10-A | *a* | " | 8 | 5.14 ± 0.08 |
| B10-Br | *k* | " | 8 | 6.40 ± 0.10 |
| RF/J | *k* | Ig-1$^c$ | 6 | 6.17 ± 0.17 |
| DBA/2N | *d* | Ig-1$^c$ | 11 | 6.38 ± 0.19 |
| AKR | *k* | Ig-1$^d$ | 5 | 5.23 ± 0.26 |
| A/J | *a* | Ig-1$^e$ | 6 | 6.54 ± 0.06 |
| CE/J | *k* | Ig-1$^f$ | 10 | 6.48 ± 0.12 |

All mice were bled 12 days after the second injection of NBrP-CG.

* Log means ± standard error.

vious paper (8) are alleles of the same cistron. If this were true, then the BALB/c allele is dominant in NBrP immunization but the C57BL/6 allele is dominant in NP immunization (unpublished experiments). C57BL/6 and SJL/J strains produce anti-NP antibodies of different but anti-NBrP antibodies of indistinguishable fine specificity types. This weakly suggests that the NP and the NBrP markers are not alleles of one cistron since both strains are homozygous. The evidence is inconclusive, however, only a meiotic recombination between the two markers could demonstrate that they belong to different cistrons, while true allelism is impossible to prove.

There are at least four types of Mendelian genes that control the magnitude of specific immune responses. One type includes the *H-2*-linked *Ir* genes controlling antigen recognition by thymus-derived lymphocytes (18, 19). The second type is exemplified by the data of Blomberg et al. (6) and by the data presented above. When *Ir* genes of this type are being studied without influence of other genes the magnitude and the fine specificity (affinity) are intimately connected. The third type was demonstrated by antigen-specific responsiveness or unresponsiveness that was controlled by one Mendelian gene unlinked to the *H-2* and to allotype (20, 21). The fourth type is manifested by a simultaneous low or high response to several antigens (22, 23). It is genetically controlled but several genes are involved.

## Summary

Mice of 17 inbred strains produced anti-(4-hydroxy-5-bromo-3-nitrophenyl)-acetyl (NBrP) of three different fine specificity types. Anti-NBrP antibodies of all allotype b mice (five strains tested) had a high relative affinity for (4-hydroxy-3.5-dinitrophenyl)acetyl (NNP) but low for (4-hydroxy-5-cloro-3-nitrophenyl)acetyl (NClP). Another category was characterized by high relative affinity for NClP but low for NNP. This category included most of the tested strains. The third category (CBA and C3H strains) had an intermediate fine specificity. Associated with fine specificity characteristics were anti-NBrP titers, mice of allotype b had lower titers than the other mice.

Studies of congenic, recombinant inbred, $F_1$ and backcross mice showed that both the fine specificity and the magnitude of the anti-NBrP response of BALB/c mice were controlled by an allotype-linked gene. This gene was dominant over the C57BL/6 allele. Lack of recombinant mice in the backcross generation on one hand and a linkage disequilibrium between allotypes and fine specificity patterns on the other suggest close linkage between the two genes.

*Received for publication 19 December 1974.*

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