

INHERITANCE OF ANTIBODY SPECIFICITY
III. A New V_H Gene Controls Fine Specificity of
Anti-*p*-Azobenzenearsonate Coupled to the Carbon Atom 5 of
Hydroxyphenylacetic Acid in the Mouse*

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Variable (V)¹ genes code for the variable polypeptide sequences of immunoglobulin molecules. Available evidence indicates that there are three pools of V genes in most mammalian haploid sets of chromosomes. One pool is shared by all kappa chains, another by lambda chains, and the third by all heavy chain classes and subclasses. The three pools are called V_K , V_λ , and V_H genes.

V_H genes of the rabbit and the mouse have been defined on Mendelian terms (1). All mouse V_H genes have been found to control one kind of antibody only. At least six such genes have been found (2-7) and five have been roughly mapped on the basis of one or two crossover strains (8-11).

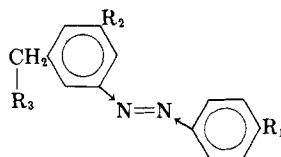
The methods used for the demonstration of these V genes included antigenic characterization [classical idiotypes, (12, 13)], isoelectric focusing, or fine-specificity characterization. Since all these methods probably detected closely similar V domains and the choice of the method does not matter from the genetic point of view, we use the word idio type in this paper to characterize the product of one Mendelian V gene. In this paper we report a new V_H gene controlling antibodies to a hapten of *p*-azobenzenearsonate coupled to the carbon atom 5 of hydroxyphenylacetic acid (ABA-HOP) (Fig. 1).

Materials and Methods

Mice. All animals used in this study were bred in our laboratory. Their origins were: CBA/H were obtained from the National Institute for Medical Research, London, England, in 1964; strains C57BL/6 and BALB/c were obtained from the Jackson Laboratory, Bar Harbor, Maine, in 1965 and 1968, respectively; and the other strains from the same laboratory in the years 1972, 1973, and

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¹ *Abbreviations used in this paper:* ABA-HOP, *p*-azobenzenearsonate coupled to the carbon atom 5 of HOP; ABA-MIP, ABA coupled to the carbon atom 5 of (4-hydroxy-5-iodophenyl)acetyl; ABA-NP, ABA coupled to the carbon atom 5 of (4-hydroxy-3-nitrophenyl)acetyl; ABA-TYR, ABA-L-tyrosine; ABS-HOP, *p*-azobenzene sulfonate coupled to the carbon atom 5 of HOP; Boc-TYR, butyloxycarbonyl tyrosine; BSA, bovine serum albumin; HOP, hydroxyphenylacetic acid; HVE, high-voltage electrophoresis; IC_{50} , hapten concentration causing 50% inhibition of a serological reaction; K_{rel} , relative affinity; NBrP, (4-hydroxy-5-bromo-3-nitrophenyl)acetyl; PFU, plaque-forming unit; TLC, thin-layer chromatography; V , variable.



	R ₁	R ₂	R ₃
ABA-HOP	ASO ₃ H ₂	H	COOH
BOC-ABA-TYR	ASO ₃ H ₂	H	BOC-NHCHCOOH
ABA-NP	ASO ₃ H ₂	NO ₂	COOH
ABA-MIP	ASO ₃ H ₂	I	COOH
ABS-HOP	SO ₃ H	H	COOH

FIG. 1. The haptens used. The compounds are coupled to proteins via the carboxyl groups in R₃.

1974. Mice were vaccinated against ectromelia at the age of 3–5 wk, and they entered this study at the age of 3–7 mo.

Haptens and Their Conjugates. The haptens used are described in Fig. 1. *p*-aransilic acid and 4-hydroxyphenylacetic acid (HOP) were purchased reagent grade, and recrystallized from hot water. Butyloxycarbonyl tyrosine (Boc-TYR) was reagent grade (Sigma Chemical Co., St. Louis, Mo.).

Boc-ABA-TYR. Formed by reacting the diazonium salt derived from *p*-aransilic acid with Boc-TYR according to the method of Tabachnik and Sobotka (14). The desired product was purified and analyzed as previously described (15). Briefly, after the diazotization, Boc-ABA-TYR was precipitated by HCl (pH 3), redissolved in a 0.1 M ammonium carbonate buffer (made 0.01 M in pyridine, pH 10, and saturated with ethyl acetate), and purified on a LH-20 Sephadex column equilibrated in the same buffer. The appropriate orange-red peak was collected, and acidified to pH 3.0. The resulting precipitate was extracted into ethyl acetate. The ethyl acetate was dried by addition of sodium sulphate, and the desired product crystallized after addition of ether. Purity was indicated by thin-layer chromatography (TLC) and high-voltage electrophoresis (HVE).

ABA-HOP. HOP was diazotized and purified up to crystallization as for Boc-TYR. ABA-HOP was crystallized from boiling methanol. This product was pure (TLC and HVE) having an arsenic to nitrogen ratio (neutron activation analysis) corresponding to theoretical (theoretical 2.68; experimental 2.70).

ABA Coupled to the Carbon Atom 5 of (4-Hydroxy-5-Iodophenyl)Acetyl (ABA-MIP). ABA-MIP was synthesized from ABA-HOP by iodination with chloramine-T (16). ABA-HOP (2 mm) was dissolved in 75 ml of 0.1 M borate buffer, pH 8.6, containing 5 mm potassium iodine. 2.5 mm chloramine-T was then added, the reaction mixture was extracted with chloroform to remove excess molecular iodine, brought to pH 3 with HCl, and the desired compound extracted with ethyl acetate and dried by addition of sodium sulfate. The product was crystallized in ethyl acetate by addition of *n*-hexane. ABA-MIP was pure on HVE pH 6.5 (pyridine/acetic acid/H₂O, 400:16:3,600) and found to have a slower mobility than ABA-HOP. The visible spectrum was identical to ABA-HOP. When monoiodo-HOP was diazotized with arsanilic acid the resulting compound had identical electrophoretic and spectral properties.

ABA Coupled to the Carbon Atom 5 of (4-Hydroxy-3-Nitrophenyl)Acetyl (ABA-NP). NP was diazotized as was ABA-HOP, and initially purified in a similar manner on LH-20 Sephadex. The third peak (bright red) was extracted with ethyl acetate after acidification to pH 3.0 and precipitated with petroleum ether. 150 mg of this semipure material was placed on a 5 × 50 cm DEAE-Sephadex column equilibrated in 0.05 M Tris-HCl buffer, pH 8.6. The material was eluted employing a 0.1–0.3 M NaCl gradient in the same buffer. The second peak was found to be pure by HVE and TLC. The mobility on HVE was slightly greater than ABA-HOP. The spectrum in the visible range was similar to that of ABA-HOP except showing a hyperchromatic shift of the 490 nm

TABLE I
Titers and IC₅₀ Values of Anti-ABA-HOP Antibodies from Different Strains of Mice

Strain	Allo- type	No. of mice	Titer	IC ₅₀ concentration with:				
				ABA- HOP	Boc- ABA- TYR	ABA- NP	ABA- MIP	ABS- HOP
C3H	a	7	5.042 ± 0.240	14			71	<u>333</u>
CBA	a	14 (9)	5.500 ± 0.138	8.5	13	390	24	<u>310</u>
BALB/c	a	19 (12)	4.726 ± 0.190	13	18	110	<u>2.6</u>	8,200
C57BL/6	b	16 (10)	4.644 ± 0.161	25	35	120	<u>1.3</u>	7,600
SJL/J	b	7	5.094 ± 0.152	19	23		<u>3.3</u>	13,000
A/J	e	13 (8)	5.395 ± 0.108	16	11	<u>9,400</u>	<u>360</u>	23,000
AKR/J	d	8	NT	73	97		<u>490</u>	
CE/J	f	7	5.432 ± 0.165	31	26		41	15,000
P/J	h	7	NT	41			55	
DBA/2J	c	14 (9)	4.331 ± 0.141	41	50	200	<u>11</u>	21,000

Titer ± standard error is the log mean of the 50% PFU inactivation titers of sera taken 20 days after the first antigen injection. IC₅₀ values give the nanomolar concentration of the hapten required to reduce haptenated phage inactivation titer to 50%. They are geometric mean values. Numbers in parentheses indicate the number of mice tested with ABA-NP and ABS-HOP. These compounds were not used in all tests. Underlined and boxed means differ significantly ($P < 0.05$) from all other means of the column, except from those underlined by a broken line. The sera used for hapten inhibition studies were bled 15 days after the second injection of ABA-HOP-BSA.

peak to 500 nm with an $\epsilon_M = 13,640$, compared to 10,500 for ABA-HOP. Ratio of arsenic (neutron activation analysis) to nitrogen (Dumas) was as expected (theoretical 1.9; experimental 1.8).

p-Azobenzene Sulfonate Coupled to the Carbon 5 Atom (ABS)-HOP. For the synthesis and purification the same methods were used as for the synthesis and purification of ABA-HOP.

Antisera. Mice were injected twice with 50 μ m of ABA-HOP_{7.3} bovine serum albumin (BSA). Each injection was given in complete Freund's adjuvant (into four sites subcutaneously), on days 0 and 40. Mice were bled on days 20 and 55. The sera were stored below the temperature of -20°C .

Antibody Titrations. Anti-ABA-HOP titers were determined by the inactivation of Boc-ABA-TYR-T4 bacteriophage as was originally described for (4-hydroxy-5-iodo-3-nitrophenyl)acetyl-T2 phage (17). This haptenated phage was used since we could not prepare an ABA-HOP phage conjugate of a corresponding sensitivity, and since anti-ABA-HOP appears to have approximately the same affinity for Boc-ABA-TYR as for ABA-L-tyrosine (ABA-TYR) (Table I). From Fig. 1 we see that the terminal and the pentaminal benzene rings have identical substitutions in these two compounds. Preparation of these phage conjugate has been described previously (18). ABA-MIP-T4 phage was used in some experiments. Its preparation was analogous to the preparation of the ABA-TYR-T4 phage. 20 mg of N-hydroxysuccinimide were dissolved in 0.8 ml methanol and incubated at room temperature for 90 min. 2.4 ml of 3% NaCHO₃ was added, the resulting precipitate was quickly removed, and varying volumes of the filtrate were added to 1.2-ml batches of T4 phage (10 plaque-forming units [PFU]/ml of 3% NaHCO₃).

Determination of Fine Specificity. For this purpose we conducted hapten inhibition studies with the immunogenic hapten ABA-HOP and with four structurally related haptens. The 50% inhibiting concentration (IC₅₀) for each hapten was determined as previously described (19, 22). This (interpolated) concentration would reduce antibody activity to the level of twice diluted antibody. From the five IC₅₀ values four relative affinities were calculated for each cross-reactive hapten according to the formula:

$$K_{rel} = \frac{\text{IC}_{50} \text{ for the homologous hapten}}{\text{IC}_{50} \text{ for the heterologous hapten.}}$$

In an experiment of this type (Fig. 3) 0.4-ml samples of an anti-ABA-HOP serum from (C57 × A/J)F₁ diluted 1/30,000 were distributed into 51 tubes. Another 0.4 ml was added into each tube, this volume contained a known concentration of one of two haptens or broth (three tubes). The three tubes were uninhibited antibody controls (100% control). Into three other tubes containing 0.4 ml broth we added 0.4 ml of the same serum diluted 1/60,000 (50% control). In this experiment we had additional tubes containing 0.4 ml of broth. Into three of them we added 0.4 ml of the serum diluted 1/150,000 (20% control); for three others, serum dilution 1/120,000 was used (25% control). For three other tubes dilution was 1/90,000 (33% control), for three others 1/45,000 (67% control), and so on. The tubes were stirred and 0.4 ml of broth containing ca. 1,000 PFU of ABA-HOP-T4 was finally added. The tubes were incubated at +37°C for 5 h, and the contents were then plated with bacteria. After an overnight incubation plaques were counted. Serum dilution 1/30,000 was used in all hapten-containing tubes. Without an inhibition this antibody concentration reduced the constant starting number (1,200) of Boc-ABA-TYR-T4 PFU to the average of 2.7. Serum dilution 1/60,000 (50% control) left 21.3 surviving PFU. Serum dilution 1/90,000 (33% control) left 44.3 survivors, dilution 1/120,000 (25% control) 89 survivors, and 1/150,000 (20% control) 147 survivors. ABA-HOP (circles) and ABA-MIP (dots) were used as inhibitors in different concentrations. IC₅₀ values for ABA-MIP and ABA-HOP were 10⁻⁸ and 4.7 × 10⁻⁸, respectively, but the former curve is doubly sigmoid indicating a majority idiootype with an ABA-MIP IC₅₀ value <10⁻⁸ M and a minority idiootype (~30%) with an IC₅₀ value between 10⁻⁶ and 10⁻⁵ M. The former type is heteroclitic antibody (20).

Antiallotype Sera. They were raised by immunizing mice of one strain with complexes of *Bordetella pertussis* bacteria and anti-*Bordetella* of another strain (21). Anti-a was raised by immunizing C57BL/6 mice with bacteria-coated BALB/c antibody. Anti-b was produced by a reciprocal arrangement and anti-e by immunizing BALB/c mice with A/J antibodies. This anti-e did not react with C57BL/6 sera.

Results

Quantity of Anti-ABA-HOP and Its Affinity (IC₅₀ value) for Different Haptens. The mean titers varied from strain to strain (Table I). The differences were greater in the primary than in the secondary response, and the primary response titers are given in the table. CBA, A/J, and CE were high responders, C57BL/6, BALB/c, and DBA/2 were low responders.

Fine specificity of the anti-ABA-HOP antibodies was tested by inhibition with five structurally related haptens including ABA-HOP, the hapten used as immunogen. The 50% inhibitory concentration (IC₅₀) of ABA-HOP was a little over 10⁻⁸ M in all tested strains of mice (Table I). IC₅₀ values for Boc-ABA-TYR were only slightly higher than the values for ABA-HOP. The other three compounds revealed at least four fine-specificity types in 10 strains of mice. One type was produced by the C3H and the CBA mice, and it is characterized by a high affinity for ABS-HOP (mean IC₅₀ values in all other strains was more than 25 times higher than in C3H and CBA). The second type of antibody was characterized by a high affinity for ABA-MIP. The model strain was C57BL/6 but BALB/c and SJL mice did not differ significantly from it. These strains had heteroclitic antibody, its affinity for ABA-MIP was higher than for the immunogen ABA-HOP.

The third model strain is A/J whose anti-ABA-HOP had a low affinity for ABA-MIP and ABA-NP. The strains CE and P must be put in one type at this time, they differ from the three types mentioned above but not necessarily from each other. DBA/2 may belong to a fifth type.

Inheritance of the Fine Specificity of Anti-ABA-HOP. We bred four types of F₁ hybrids and produced backcrosses to one or both of the parents. All hybrids were immunized with ABA-HOP-BSA. Fine specificity of the antihapten anti-

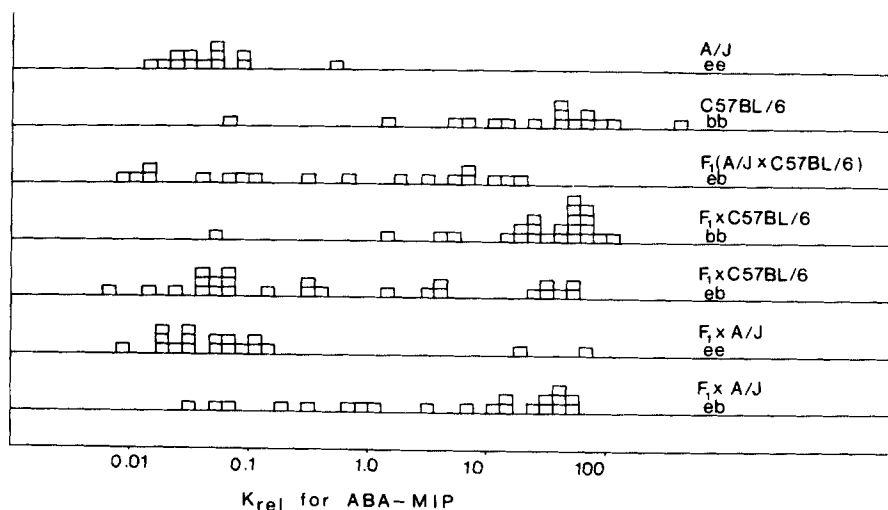


FIG. 2. Inheritance of antibody fine specificity in crosses between C57BL/6 and A/J mice. Each square in the picture represents a mouse whose genotype is given on the right-hand margin. Their position in the horizontal direction indicates the relative affinity of their anti-ABA-HOP for ABA-MIP $[(IC_{50} \text{ ABA-HOP})/(IC_{50} \text{ ABA-MIP})]$.

bodies of the secondary response was determined by the hapten inhibition tests. Since greatest strain differences could be detected with the aid of ABA-MIP only this hapten and ABA-HOP were used in the study of the hybrid mice. The relative affinity (K_{rel} value) for ABA-MIP was obtained by dividing the IC_{50} value of ABA-HOP by the corresponding IC_{50} value of ABA-MIP.

Studies in A/J and C57BL/6 mice included the parents, the F_1 hybrid, and backcrosses to both parents. They had different types of anti-ABA-HOP (Fig. 2). Anti-ABA-HOP of all but one C57 mouse was heteroclitic for ABA-MIP. With this exceptional mouse we determined complete inhibition curves (exemplified in Fig. 3), and found this serum to contain two distinguishable populations of antibody, a larger population with a K_{rel} of about 0.07 and a smaller population with a K_{rel} value of ca. 7. Even this mouse had heteroclitic antibody although as a minority population. All A/J mice had relative affinities less than 0.6, and none of them had a heteroclitic minority population.

Most $(A \times C57BL/6)F_1$ animals had intermediate K_{rel} values which varied more than the values of the parent strains. The great scatter of F_1 mice was caused by a variation in the IC_{50} values for ABA-MIP rather than in the ABA-HOP values. Some of these mice exhibited doubly sigmoid ABA-MIP inhibition curves of the type illustrated in Fig. 3. They probably contained a mixture of both parental idiotypes.

The $F_1 \times C57$ backcross mice closely resembled the C57 parent if they had inherited both Ig-1 haplotypes from the C57 parent (b/b) but were like the F_1 hybrids if they had inherited one Ig-1 allele from each parent. The backcross mice from the cross $F_1 \times A/J$ behaved in a corresponding manner; e/e homozygotes greatly resembled the A/J parents and e/b heterozygotes had an intermediate distribution.

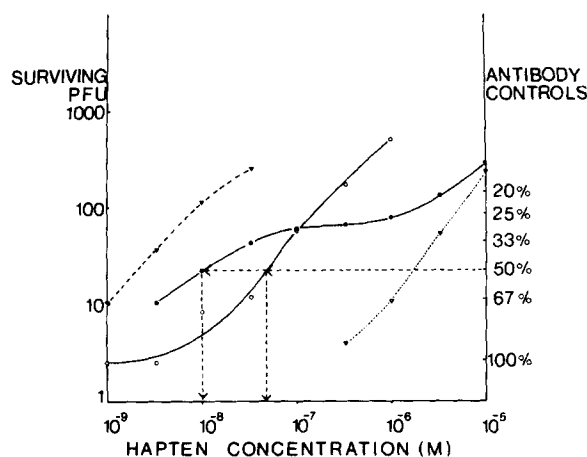


FIG. 3. Quantitation of two idiotypes of an anti-ABA-HOP serum with the aid of hapten-inhibition curves. The serum of a (C57 \times A/J) F_1 mouse was studied (solid lines). Inhibition by ABA-MIP of an b/b homozygote antibody (broken line) and e/e homozygote antibody (dotted line) are given for a comparison. The control values on the right-hand margin are valid for the b/e serum but the 50% control values for the other sera were similar (25 and 28.5).

Crosses between strains A/J and BALB/c included the F_1 hybrid and its backcross to strain A. F_1 animals had a greater scatter than either parental strain, and the average K_{rel} was intermediate between the parents (Fig. 4). The fine specificity of anti-ABA-HOP in the $F_1 \times$ A/J backcross mice again was dependent on the allotype. Homozygous (e/e) mice had a distribution indistinguishable from that of the A/J parents. Those heterozygous for the allotype (a/e) were indistinguishable from the F_1 hybrids (Fig. 4). The distributions of the two backcross populations were significantly different ($t = 4.9$, and $P < 0.001$).

Crosses between C57BL/6 and CBA strains included the F_1 hybrid and a backcross to CBA. All but one of the C57 antisera were heteroclitic for ABA-MIP whereas all but one of the CBA antisera were conventional. Of the 20 F_1 hybrids tested 6 were in the typical CBA area ($K_{rel} < 0.8$), 10 were in the typical C57 area ($K_{rel} > 4$) and 4 had intermediary values. Most of the intermediate mice had two distinguishable antibody populations since the ABA-MIP inhibition curves were doubly sigmoid (cf. Fig. 3). The C57 haplotype thus looks slightly dominant with ca. 60% penetrance (Fig. 5).

Out of 56 mice of the $F_1 \times$ CBA backcross progeny 32 were homozygous for the CBA allotype a and 24 were a/b heterozygotes. The relative affinities for ABA-MIP of the former group had a distribution closely resembling the distribution of CBA mice, and the distribution of the heterozygotes resembled that of the F_1 hybrids. These two groups differed significantly from each other ($t = 3.367$, and $P < 0.005$).

We had six backcross mice from a cross (AKR \times C57BL/6) \times AKR and tested their anti-ABA-HOP. The results (Table II) are inconclusive but they suggest that a dominant or codominant allotype-linked gene controls the heteroclitic idiomorph also in this cross.

Inheritance of Antibody Titers. Of the four strains that entered the breeding

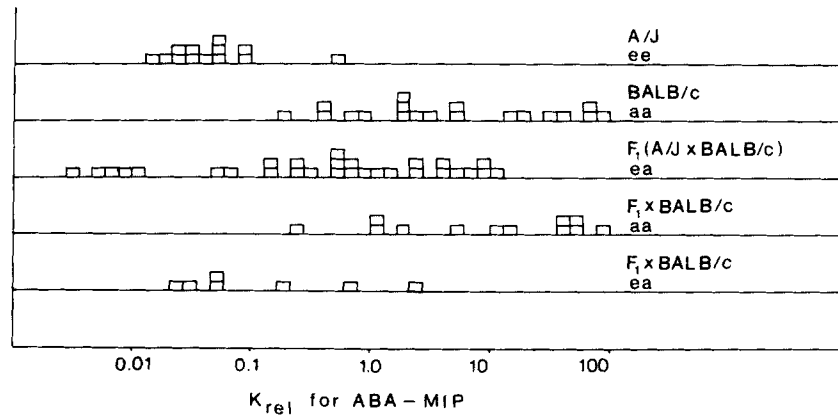


FIG. 4. Inheritance of antibody fine specificity in crosses between BALB/c and A/J mice. For explanations see Fig. 2.

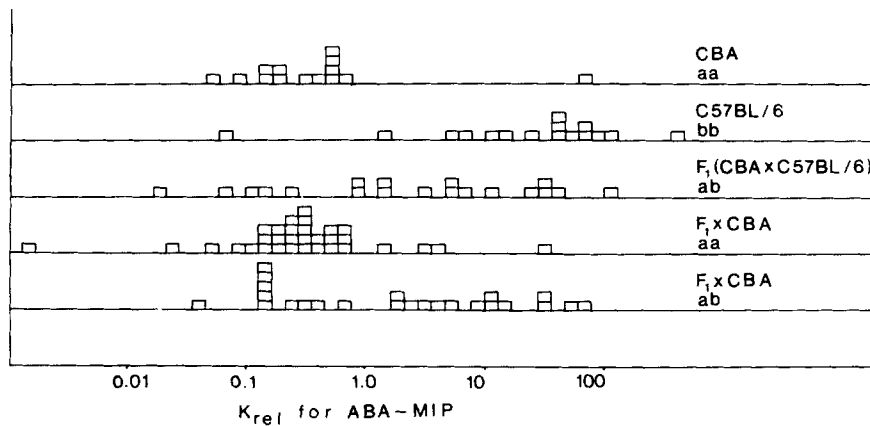


FIG. 5. Inheritance of antibody fine specificity in crosses between C57BL/6 and CBA mice. For explanations see Fig. 2.

experiments two (CBA and A/J) were high responders and two (C57BL/6 and BALB/c) were low responders (Table I). Titers of various hybrid mice are given in Table III. Backcross mice were alltyped but we could detect no statistically significant allotype-linked differences in titer. For this reason mice were not divided according to the allotype in Table III. Female mice tended to have slightly higher titers than males in most groups (cf. 23) but no bimodal distribution could be detected in any of the 12 genealogically different groups of mice. Thus they were kept undivided.

The (CBA x C57BL/6) F_1 hybrids had a mean titer intermediate between the parental titers. The F_1 x CBA backcross progeny had a mean titer intermediate between its parents but close to the F_1 titer.

Members of the crosses between A/J and C57BL/6 behaved in an analogous manner. The F_1 hybrids had an intermediate position between the parental

TABLE II
Inheritance of Fine Specificity in the Cross between AKR and C57BL/6 Strains

Strain (allotype)	No. of mice in the following ranges:					Total
	0.01-0.1*	0.1-1.0	1.0-10	10-100	100	
C57BL/6 (bb)	1	—	3	10	2	16
AKR (dd)	3	5	—	—	—	8
(AKR × C57) × AKR	(bd) —	—	1	1	—	2
Backcross	(dd) 3	1	—	—	—	4

* These figures are relative affinities of anti-ABA-HOP for ABA-MIP.

TABLE III
Inheritance of Anti-ABA-HOP Titers

Genotype	No. of mice	Mean log titer	SE
BALB/c	20	4.73	0.19
(BALB × A) × BALB	28	4.86	0.09
(BALB × A)F ₁	28	5.44	0.07
(BALB × A) × A	9	4.92	0.12
A/J	12	5.39	0.11
(A × C57) × A	27	5.18	0.08
(A × C57)F ₁	18	5.00	0.10
(A × C57) × C57	49	4.56	0.06
C57BL/6	15	4.64	0.16
(C57 × CBA)F ₁	20	4.99	0.11
(C57 × CBA) × CBA	66	5.02	0.06
CBA	14	5.50	0.14

Sera were bled on day 20 after an injection of the antigen.

mice. Backcross mice to the high responder parent resembled the F₁ hybrids whereas the backcross to the low responder produced low responders.

The offspring of the BALB/c and A/J presented a different picture. The F₁ hybrid mice were as good responders as the A/J mice but the backcross to the low BALB/c produced low responders. The backcross to the A/J produced mostly low responders but three out of nine animals were in the A/J range.

Discussion

Anti-ABA-HOP antibodies of different mouse strains had greatly different fine specificities demonstrable by hapten inhibition studies. At least four fine-specificity types of anti-ABA-HOP could be distinguished using five chemically related haptens (Table I). In two mouse strains a strain-specific isoelectric-focusing pattern could also be demonstrated in this antibody (K. Karjalainen, personal communication). We use the word *idiotypic* for these types. The model *idiotypic* of a strain was predominant in the antibody of most but not all mice.

In F₁ hybrid animals between idiotypically different parents we often found a

mixture of the two parental idiotypes. Such a mixture caused doubly sigmoid inhibition curves with ABA-MIP since one idiotypic had ca. 100 times higher affinity for ABA-MIP than the other. The two steep portions of the curve corresponded to each of the two idiotypes, and from the level of the intermediate plateau the proportions of the two idiotypes could be roughly estimated.

These idiotypes were inherited as allotype-linked Mendelian traits in four breeding experiments. The simplest explanation for our data is that few germ-line cistrons serve as structural genes for the V_H polypeptides of mouse anti-ABA-HOP. These polypeptides predominantly control the fine-specificity characteristic that we measured, relative affinity for ABA-MIP. Other genes including the V_L genes would have little effect on this characteristic. We must further assume that there are at least three different sets of cistrons, one in the A/J strain, another in C57BL/6 and BALB/c, and the third in CBA. We do not know whether the V_H -ABA-HOP cistron(s) of the A/J mice takes a place in the chromosome that is analogous to the place of the C57 cistron(s) (true allelism). Indeed it is uncertain whether the word true allelism is applicable for the V regions of the chromosome since chromosomal rearrangements may be very frequent in this area. Even the length of this area can differ in different strains of mice.

The ABA-HOP antibody is unique in that several different V-gene alleles can be distinguished and many breeding programs performed. This will give us the first opportunity to compare the map position of two idiotypic alleles controlling the same antibody. Tentative evidence obtained from a cross-over strain (8) suggest that the V_H -ABA-HOP gene of the BALB/c is far from the allotype gene, probably further away than the V_H -dex (11) or V_H (4-hydroxy-5-bromo-3-nitrophenyl)acetyl (NBrP) genes (Mäkelä and Eichmann, unpublished observation). By studying recombinants between A/J and C57BL/6 it will also be possible to test whether in the C57BL/6 strain the V_H -ABA-HOP gene is far away from the allotype gene.

Three exceptional mice were found in the crosses between A/J and C57 mice. One b/b mouse from the $F_1 \times C57$ backcross had an unexpectedly low K_{rel} value for ABA-MIP and two e/e mice had a high value. A possible crossing over event could have created the new combinations of an idiotypic and allotype. Another possibility is plausible in the first case; the mouse perhaps was an analogue of the exceptional C57 mouse shown in Fig. 2. Crossing over is a more likely explanation for the remaining two mice since no exceptional A/J mice have been found. Breeding studies will be necessary to establish the existence of cross-over chromosomes but so far the data are compatible with a high recombination frequency between the allotype and the V_H -ABA-HOP gene of the C57BL/6. The data provide no evidence against true allelism of the BALB/c and C57BL/6 genes.

All three types of F_1 hybrid strains expressed both parental idiotypes in approximately equal proportions when taken as populations. In many individual F_1 mice, however, only one or the other parental idiotypic could be detected. This situation suggests that all the detected idiotypes are almost equal in antigenic selection. In two previously reported cases it was not so, the BALB/c haplotype was clearly dominant over the C57BL/6 haplotype in the response to a

NBrP immunization (7), and the C57BL/6 haplotype almost dominated over the CBA haplotype in anti-NP of most F₁ hybrids (6).

The fact that one parental idiootype was predominant in some but the other idiootype in other F₁ mice can be easily explained if the ABA-HOP-sensitive B lymphocytes of an individual mouse arise from a small number (say three) of antigen-committed precursor cells. In this case the haplotype of one parent might accidentally be expressed in most antigen-reactive B cells. A less plausible explanation is that only few B cells responded to the antigen in at least some mice. These cells would then have had to expand into big clones to explain all the antibody formation of a mouse.

We found no evidence that a polymorphism in L-chain genes would contribute to the inherited idiotypes of anti-ABA-HOP. Particularly impressive was the fact that e/b backcross mice having 75% of their genes (including L-chain genes) from A/J parents were not more A/J-like than e/b mice with only 25% of their L-chain genes originating from the A/J parents (Fig. 2). Another finding pointing in the same direction was our failure to find new idiotypes in hybrid animals. Hybrid animals often had values intermediate between parents but usually we found evidence for a mixture of the parental idiotypes rather than a new idiootype.

Lack of genetic markers in the V_L polypeptides is strange since the number of known V_H markers is now ca. 15. Since both the V_L and the V_H polypeptides contribute to the combining site (24, 25) and since the variability of both the V_L and the V_H correlate with antibody specificity (26, 27) similar numbers of Mendelian V genes would have been expected for both polypeptides. A possible explanation is that somatic mutations are important in the generation of mouse L-chain diversity whereas diversity of the H-chain is more due to germ-like variability.

Importance of the V_H genes is easier to explain in cases where an inherited idiootype is connected with one of few very similar H-chain sequences but several less similar L-chain sequences.² It is more difficult to explain in cases where an inherited idiootype appears to require a certain sequence both in the H and in the L chains. (28).

Pawlak and his co-workers found an inherited idiootype in antihapten antibodies produced by A/J mice to protein conjugates of azobenzene arsonate (3). In these conjugates most of the hapten is attached on tyrosine residues, and the resulting structure resembles ABA-HOP. The structures are not identical, however, since our "antigen" is predominantly ABA-HOP-lysine whereas their antigen is predominantly ABA-TYR. Lysine and tyrosine are residues of the carrier protein. For two reasons we believe that the two antigens stimulate different clones in A/J mice. One reason is that ABA-Boc-TYR-lysine did not elicit the heteroclitic idiootype in BALB/c mice (unpublished observations). Another reason is a preliminary observation made together with K. Eichmann, that the BB δ 7 recombinant chromosome (8) probably contains the ABA-HOP

² Capra, J. D., A. S. Tung, and A. Nisonoff. Structural studies on induced antibodies with defined idiotypic specificities. II. The light chains of anti-*p*-azophenylarsonate antibodies from A/J mice bearing a cross-reactive idiootype. Manuscript in preparation.

gene of the A/J strain, whereas it contains the Ars gene of the BALB/c parent.

In some earlier cases not only linkage could be demonstrated between a V_H gene and allotype genes but also association between certain allotype alleles and V_H alleles in a collection of inbred strains. For instance, NP-(4-hydroxy-3,5-dinitrophenyl)acetyl affine anti-NBrP antibody was produced by most allotype b strains but not by other strains of mice (7). Such an association could not be shown in the ABA-HOP polymorphism. CBA and BALB/c, both allotype a strains, had clearly different idiotypes. A/J and NZB/BL, both allotype e strains, probably have different idiotypes (unpublished observations). On the other hand, BALB/c and C57BL/6 had indistinguishable idiotypes but different allotypes. Lack of an association between allotypes and idiotypes in anti-ABA-HOP antibodies may be connected with the probable long distance between the corresponding loci.

Not only did different strains produce different kinds of antibody but the titers were also different. CBA, A/J, and CE strains had 20-day titers ranging from 5.40 to 5.50 whereas DBA, BALB/c, and C57BL/6 strains had mean titers less than 4.80. From earlier work we knew that these low titers were not due to general immunological inferiority, especially the BALB/c mice responded strongly to haptens NP and NBrP. Since other Mendelian V genes have been shown to control not only the quality but also the intensity of the response to a certain antigen (2, 7) we expected these titer differences to be due to different V genes. The breeding experiments did not confirm this expectation. We tested three crosses between a high- and a low-responder strain, and in no case could we demonstrate a V-gene effect.

The general conclusion from Table III is that several unlinked genes must control the antibody titers. Both backcrosses to the low strains produced low responder mice without any indication of bimodal distribution. Also the fact that backcross mice to the high parent did not have higher average titers than the F_1 hybrids speaks for a polygenic control.

The cross between the BALB/c and the A/J strain is interesting because of the large difference between the F_1 hybrid population and the $F_1 \times$ BALB/c population. Both means are derived from three independent groups of mice and the difference was highly significant. Since the F_1 hybrid population was as high as the high parent A/J, relevant A/J genes are probably dominant over the BALB/c genes. The fact that the backcross population was as low as the low parent suggests polygenic inheritance. The behavior of the $(A/J \times BALB) \times A/J$ mice is difficult to explain but this was only one group of nine, and it may have been a sick group since two of these mice died prematurely.

Summary

Mice of 10 inbred strains were immunized with a protein conjugate of a hapten of *p*-azobenzeneearsonate coupled to the carbon atom 5 of hydroxyphenylacetic acid (ABA-HOP), and anti-ABA-HOP titers were determined by the haptened phage inactivation. Mean titers of C57BL/6 and BALB/c mice were significantly lower than those of A/J and CBA strains. The titers were under a polygenic control and did not correlate with allotypes in backcross mice.

Fine specificity of the anti-ABA-HOP was characterized by inhibiting the

haptened phage inactivation reaction with five chemically related compounds including ABA-HOP (Fig. 1). This antibody was genetically more polymorphic than any other antibody studied. Three distinct idiotypes could be demonstrated and the number is probably greater.

The idiotypes of the A/J and C57BL/6 were inherited as allotype-linked dominant Mendelian traits, the former in two and the latter in three different backcrosses. Codominance of the two alleles was shown since approximately equal amounts of the two idiotypes were produced by the population of heterozygous mice. There were many individual heterozygotes, however, in which only one parental idotype could be detected. In other individuals of the same genotype the other parental idotype was predominant. In many mice a mixture of the two idiotypes was indicated by doubly sigmoid inhibition curves. Proportions of the two idiotypes could be roughly estimated from an inhibition curve. The causes for the variation in the expression of the two alleles in genotypically identical mice is discussed.

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