

*H-2*-LINKED GENETIC CONTROL OF IMMUNE  
RESPONSIVENESS TO OVALBUMIN AND  
OVOMUCOID\*

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In several recently described systems (1) immune responsiveness has been shown to be controlled by genes closely linked to histocompatibility (*H*)<sup>1</sup> loci. The first observations in this direction were made in studies of the ability of mice to respond to a series of branched, multichain, synthetic polypeptide antigens (2, 3). By the use of recombinant strains of mice it was possible to map an 'immune response gene' (*Ir-1*) on the right-hand side of the *H-2* region in the *IX* linkage group (1).

More recently, responsiveness of mice to a variety of other synthetic polypeptides (4), and less well-defined antigens, such as proteins and hapten-protein conjugates (5-8), homologous (8, 9) and heterologous (10) erythrocytes, and histocompatibility antigens (11), has similarly been shown to be controlled by genes linked to histocompatibility loci. The immune responsiveness of inbred guinea pigs to a wide array of antigens has also been shown to be under control of similar genes (12, 13).

In inbred mouse strains, high immune responsiveness to repeated low doses of bovine gamma globulin and chicken's ovomucoid-hapten conjugates were found to be associated with the alleles *a* and *k* of the *H-2* locus, whereas low immune responsiveness to these antigens was associated with *b*, *d*, and *q* alleles (6). Immune responsiveness to low doses of ovalbumin had a nearly opposite distribution (7).

The present studies were undertaken to evaluate within the same experiments the extent of the negative correlation between responsiveness to ovalbumin and ovomucoid in several inbred strains of mice, some of their F<sub>1</sub> hybrids, and backcross generations. An antigen-binding assay was used to

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<sup>1</sup> Abbreviations used in paper: ABC, antigen-binding curve; *h*, histocompatibility; *Ir-1*, immune response gene; NRS, normal rabbit serum; NRS-B, NRS in borate buffer; OA, ovalbumin; OM, ovomucoid; PCA, passive cutaneous anaphylaxis; SAS, saturated ammonium sulfate.

measure serum antibody responses in parallel with assays made by passive cutaneous anaphylaxis (PCA), used in previous experiments (6, 7). The results indicated that high immune responsiveness to ovomucoid or ovalbumin is under control of single dominant genes closely linked to specific *H-2* alleles.

#### *Materials and Methods*

*Mice.*—6–8-wk old female mice of the inbred strains of  $F_1$  hybrids shown in the tables were all obtained from the Jackson Laboratory, Bar Harbor, Maine, except for the (A/J  $\times$  SWR/J) $F_1$  and the (A/J  $\times$  C57BL/6J)  $\times$  C57BL/6J backcross generations, which were bred in our laboratory. A few experiments were conducted using *H-2<sup>b</sup>* and *H-2<sup>i</sup>* mice; these latter mice are recombinants from *H-2<sup>a</sup>* and *H-2<sup>b</sup>* genotypes (1). These animals were a generous gift of Dr. E. A. Boyse, Sloan-Kettering Institute for Cancer Research, New York 10021. CFW mice (Carworth Farms, New City, N. Y.) were used as recipients for PCA reactions.

*Antigens and Immunization.*—Five times crystallized ovalbumin (OA; Pentex Biochemical, Kankakee, Ill.) and ovomucoid (OM; Mann Research Labs. Inc., New York) were dissolved in saline, and their concentrations assayed by micro-Kjeldahl (14). Subsequently optical density determinations were used to assess protein concentrations for use in the antigen-binding assays. The  $E_{1\text{cm}}^{1\%}$  for OM was found to be 4.7; that for OA was taken as 7.4. The preparations of OA and OM were tested for cross-contamination; no precipitin lines were formed in gel diffusion tests when potent anti-OA rabbit antisera were run against OM; no PCA reactions were elicited by OM in mice sensitized with anti-OA sera, and vice versa.

The adjuvant used was  $\text{Al}(\text{OH})_3$ , prepared as previously described (15), and was mixed with solutions of the immunogens immediately before intraperitoneal injection. Each mouse received 0.1 or 1.0  $\mu\text{g}$  of antigen mixed with 1 mg of  $\text{Al}(\text{OH})_3$  in 0.5 ml of saline.

All animals received two injections of the same antigen-adjuvant mixture, given 4 wk apart. They were bled at 14 and 28 days after the primary injection, and again 7 days after the secondary injection. Samples of 0.2 ml of blood were collected from each animal by orbital puncture with calibrated micropipettes (H. E. Pedersen, Copenhagen, Denmark) and diluted in 0.4 ml of saline. The resulting dilution of serum was taken as a 1:5 dilution. A small aliquot of each individual serum was pooled within each strain and bleeding, and the pools were used for PCA assays; antigen-binding assays were conducted on each individual serum dilution. At 8 wk after the secondary immunization, the mice were boosted with a large dose of antigen (1 mg of OM or 0.1 mg of OA) with 5 mg of  $\text{Al}(\text{OH})_3$  and exsanguinated 1 wk later. Before boosting the mice were injected with 2 mg/kg of Cyproheptadine (Merck Sharp & Dohme, West Point, Pa.) to protect from anaphylactic shock. The sera obtained were serially diluted and used to derive standard curves for the antigen-binding assays.

*PCA Reactions.*—Were done as previously described (15) in CFW mice using 2-hr sensitization periods to assay  $\text{IgG}_1$  antibodies and 48-hr sensitization periods for  $\text{IgE}$  (reaginic) antibodies. Reactions were elicited by intravenous injection of 0.2 mg of either OM or OA in 0.2 ml of 0.5% Evans blue dye (Eastman Kodak Co., Rochester, N. Y.) dissolved in saline. Sera were tested at 1:5 dilution and, if positive, were serially diluted twofold and assayed again. The titers shown are the highest dilutions evoking threshold reactions (5 mm diameter).

*The Farr Test.*—Was performed essentially as described by Mitchison (16). The normal rabbit serum (NRS) concentration in the diluent was increased to 20% and that in the antigen solution to 10% to increase the amount of precipitate formed in the presence of 40% saturated ammonium sulfate (SAS). This prevented loss of precipitate on decanting the supernatants which sometimes happened when lower protein concentrations were used at this concentration of SAS.

0.25 ml volumes of mouse antiserum dilutions starting at 1:5 were further diluted sixfold

in 20% NRS in borate buffer (NRS-B). 0.25 ml volumes of OA-<sup>125</sup>I or OM-<sup>125</sup>I iodinated at the level of 2-3 atoms I/molecule and 0.5 mCi/mg by the iodine chloride method (17) in 10% NRS-B were added to a final concentration of  $1.4 \times 10^{-5}$   $\mu$ moles/ml antigen-antibody mixture. After mixing and standing overnight at 4°C, 0.5 ml 80% SAS were added, the contents of the tubes were thoroughly mixed on a Vortex mixer Scientific Industries, Inc., Springfield, Mass., and the tubes were then centrifuged for 1 hr at 4°C, 1500 g. The supernatants were poured off, and the tubes drained on absorbent paper. The radioactivity of the precipitates was determined in a Nuclear-Chicago autogamma counter Nuclear-Chicago Corp., Des Plaines, Ill. and the per cent precipitated determined. The antigen-binding capacities of the sera were determined at the 35% binding point by reference to standard antigen-binding curves (ABC) for mouse antisera reacting with OA and OM at this concentration.

The standard sera used were obtained from two to three mice of each strain 1 wk after boosting with a large dose of antigen as described above. Antigen-binding assays were performed on twofold dilutions of each antiserum and the results were plotted on semilogarithmic paper. Between 80 and 30% the amount of antigen precipitated was approximately proportional to the log concentration of antibody, that is, between those two points the curves were approximately straight and parallel; the slope of the regression for OA was  $-97.8 \pm 8.31$  and for OM  $-94.8 \pm 5.27$ , both measured between 80 and 30% binding. Below 30% precipitation the binding was directly proportional to the antibody concentration. Values for the ABC at the 35% binding point for any given percentage of antigen precipitated from 10 to 70% were computed and tabulated for the different dilutions used. The tables were used down to 1% for the first dilution. For the sake of simplification the  $\mu$ moles  $\times 10^{-5}$  antigen bound were rounded off to whole numbers as expressed in the tables. Thus, an ABC less than  $0.49 \times 10^{-5}$   $\mu$ moles is expressed as 0.

*H-2 Typing.*—*H-2* typing of mice in the backcross generation was kindly performed at Dr. E. A. Boyse's laboratory by the dextran hemagglutination method (18) using sera specific for the *H-2<sup>a</sup>* allele.

## RESULTS

*Immune Responsiveness of Inbred Strains and F<sub>1</sub> Hybrids to Low Doses of OM and OA.*—Groups of three to four mice from 12 different inbred strains, from representative *H-2* types as well as five of their F<sub>1</sub> hybrids were immunized with 0.1  $\mu$ g of OA; similar groups were immunized with 1.0  $\mu$ g of OM. The primary and secondary immune responses as measured by antigen-binding assays are shown in Tables I and II; the results of the PCA assays are shown in Table III.

In agreement with our previous observations (7, 8) the results indicated that the immune responsiveness of the strains to low doses of protein antigens is related to their *H-2* type. In addition, the present results demonstrated a negative correlation between the ability to respond to low doses of ovomucoid and the ability to respond to low doses of ovalbumin. It may be seen that strains with the *a* and *k* alleles at the *H-2* locus were good responders to ovomucoid and relatively poor responders to ovalbumin. A nearly opposite result was observed with the strains having *b*, *d*, or *q* alleles at the *H-2* locus. These differences may be clearly seen by comparisons of the magnitude of secondary antibody responses. Significant differences were, however, already apparent

during the primary responses. Compare for instance the responses of  $H-2^a$  mice to ovalbumin with those of  $H-2^a$  or  $H-2^k$  mice. These latter strains were virtually nonresponders to ovalbumin during primary immunization, whereas the primary responses of  $H-2^a$  mice were quite intense. On the other hand, none of the  $H-2^b$ ,  $H-2^d$ , or  $H-2^q$  mice responded detectably to the primary injection of

TABLE I  
*Immune Responses of Different Mouse Strains to Low Doses of Ovalbumin and Ovomuroid as Measured by Antigen-Binding Assays. Relationship to Histocompatibility ( $H-2$ ) Type*

$H-2$ type	Mouse strains	Responses to ovomucoid (1 $\mu$ g)			Responses to ovalbumin (0.1 $\mu$ g)		
		14th	28th	7th (Secondary)	14th	28th	7th (Secondary)
$a$	A/J	0;2;0;0*	17;1;0;5	314;419;3;469	0;0;0;0	0;0;0;0	0;0;16;1
$k$	AKR/J	2;1;2;1	12;7;4;5	355;248;234;214	0;0;0;0	0;1;0;0	122;17;3;0
	CBA/J	0;0;0;1	7;3;2;5	224;130;83;321	0;0;0;0	0;0;0;0	0;9;6;2
	C3H/HeJ	0;0;0;0	0;0;1;0	125;2;125;3	0;0;0;0	11;0;0;0	0;0;0;0
	B10, BR	1;0;1;1	1;1;5;2	74;53;120;37	0;0;0;0	0;0;0;0	0;8;0;0
$b$	C57BL/10J	0;0;0;0	0;0;0;0	0;0;0;1	1;21;5;5	0;10;1;18	26;190;77;57
	A.BY	0;0;0	0;0;0	0;0;0	1;9;2	16;11;7	111;215;199
	LP/J	0;0;0	0;0;0	0;0;0	2;5;0;0	4;5;0;2	12;8;1;3
$d$	DBA/2J	0;0;0;0	0;0;0;0	0;0;0;0	0;0;0;1	0;0;0;2	0;d†;32;28
	B10.D2(new)	0;0;0;0	0;0;0;0	1;2;1;0	0;8;0	0;2;1	0;38;5
$q$	DBA/1J	0;0;0;0	0;0;0;0	0;0;1;0	52;39;44	48;68;18	296;518;268
	SWR/J	0	0	6	38;12	37;11	1245;d

\* Each figure represents the nearest whole number of  $\mu$ moles ( $\times 10^{-5}$ ) of antigen bound per milliliter of one individual mouse serum at the 35% point;  $1.4 \times 10^{-5}$   $\mu$ moles of antigen/ml present in the antigen-antibody mixtures. To derive micrograms of antigen bound per milliliter of serum, multiply ovalbumin values by 0.45 and ovomucoid values by 0.30. The results of individual mice at 14, 28, and 7-secondary days are shown in order, so that the development of the immune response in each mouse may be followed.

† d = dead.

ovomuroid, whereas several of the  $H-2^a$  or  $H-2^k$  mice responded, albeit moderately. The magnitude of the immune responses was variable among strains of the same  $H-2$  type, as well as among individual mice of the same strain.

Some of the mouse strains shown in Table I constitute congenic-resistant pairs, i.e., strains which differ from each other only at the  $H-2$  locus and close portions of the  $IX$  linkage group (19). The congenic-resistant pairs are: A/HeJ ( $H-2^a$ ) and A.BY ( $H-2^b$ ); C57BL/10J ( $H-2^b$ ) and B10.BR ( $H-2^k$ ); C57BL/10J ( $H-2^b$ ) and B10.D2new ( $H-2^d$ ). These strains responded to ovomucoid and to

ovalbumin according to their *H-2* genotype and not according to the rest of their genetic background.

Groups of six mice of five  $F_1$  hybrid strains were also studied as shown in Table II. Of these five hybrids, one ( $CAF_1$ ) was the product of a parental strain (BALB/cJ) not shown in Table I. Experiments not shown in the present paper, however, indicated that the responsiveness of BALB/cJ mice is similar to the responsiveness of other *H-2<sup>d</sup>* mice, such as DBA/2J. Four of the  $F_1$  hybrids tested resulted from crosses of a strain which reacted well to OA and poorly to

TABLE II  
*Immune Responses of  $F_1$  Hybrids of Different Strains of Mice to Low Doses of Ovalbumin and Ovomucoid. Relationship to Histocompatibility (*H-2*) Type*

Parental strains	$F_1$ hybrid	<i>H-2</i> type	Responses to ovomucoid (1.0 $\mu$ g)			Responses to ovalbumin (0.1 $\mu$ g)		
			14th	28th	7th (Secondary)	14th	28th	7th (Secondary)
C57BL/6J and A/J	B6AF <sub>1</sub>	<i>b/a</i>	0;0;0* 0;0;0	0;0;0 0;1;0	2;5;125 125;57;6	0;0;0 0;3;6	1;1;5 6;1;0	101;179;5 d;d;d‡
SWR/J and A/J	ARF <sub>1</sub>	<i>q/a</i>	0;0;0 3;0;1	0;0;3 27;6;10	5;2;235 396;256;157	24;9;5 51;25;11	51;77;28 39;98;27	546;277;260 675;472;327
BALB/cJ and A/J	CAF <sub>1</sub>	<i>d/a</i>	0;0;0 0;0;1	0;1;0 0;14;15	2;8;275 15;498;384	1;0;0 0;0;0	0;0;0 2;0;0	52;41;2 54;22;1
C3H/HeJ and DBA/2J	C3D2F <sub>1</sub>	<i>k/d</i>	0;0;0 0;0;0	3;0;0 4;0;1	112;112;5 1;d;d	0;0;0 0;0;0	0;0;0 0;0;2	10;1;8 1;0;48
C57BL/6J and DBA/2J	B6D2F <sub>1</sub>	<i>b/d</i>	0;0;0 0;0;0	0;0;0 0;0;0	1;1;6 1;1;1	41;8;17 25;22;22	13;15;14 30;24;13	129;240;455 383;193;215

\*, ‡ See Table I for explanation.

OM, with a strain that responded poorly to OA and strongly to OM. These four hybrids (B6AF<sub>1</sub>, ARF<sub>1</sub>, CAF<sub>1</sub>, and C3D2F<sub>1</sub>) responded well both to OA and OM. The fifth  $F_1$  hybrid was derived from two parental strains which both responded poorly to OM as well as to OA (C57BL/6J and DBA/2J). Accordingly, the resulting hybrid (B6D2F<sub>1</sub>) responded poorly to OM and quite strongly to OA.

The pattern of distribution of immune responsiveness to OM and OA of the different strains and the  $F_1$  hybrids is shown in Fig. 1, in which clusters of strains with *a* and *k* alleles, and *b*, *d*, and *q* alleles, are indicated. The responsiveness of the  $F_1$  hybrids may be easily compared with those of parental strains and observed to be inherited as a dominant trait.

In addition to the antigen-binding assays, the IgG<sub>1</sub> and IgE antibody content of the sera were also estimated by PCA reactions. The results shown in Table III may be compared with those of the previous tables. On the whole, there was close agreement between the magnitude of the immune responses as assayed by the two methods. In no situation was a high antigen-binding capacity found

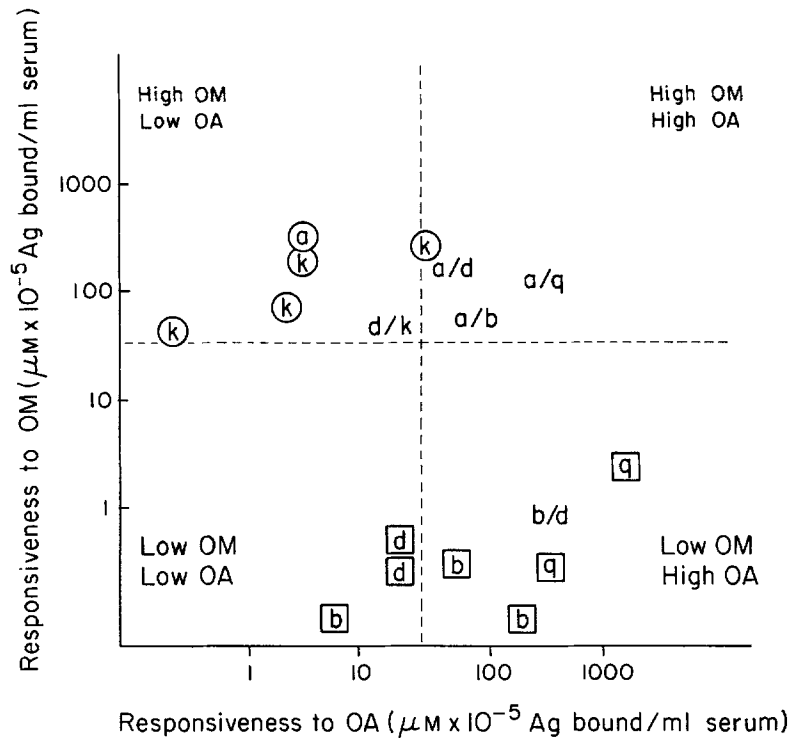


FIG. 1. Distribution of responsiveness to ovalbumin (OA) and ovomucoid (OM) as measured by antigen-binding assays: arithmetic means for the strains and their different F<sub>1</sub> hybrids. Strains with *a* and *k* alleles are enclosed in circles; strains with *b*, *d*, or *q* alleles are enclosed in squares; F<sub>1</sub> hybrids are not enclosed.

for an antiserum with low sensitizing activity, and only in very rare instances was a serum with a low antigen-binding capacity observed to have a moderate sensitizing activity. A comparison between the results obtained by antigen-binding assays and PCA reactions performed with 2-hr sensitization periods, which measure mainly IgG<sub>1</sub> antibodies, is shown in Table IV. The contribution to antigen binding by IgE antibodies was assumed to be negligible.

*Segregation of the Immune Responsiveness to OM in Linkage with H-2<sup>a</sup> Allele.*— This experiment was designed to test the linkage of immune responsiveness to low doses of OM to the H-2<sup>a</sup> allele. F<sub>1</sub> mice from crosses of C57BL/6J (H-2<sup>b</sup>)

and A/HeJ ( $H-2^a$ ) strains were backcrossed to C57BL/6J mice, and the animals of the backcross generation were tested both for their responsiveness to OM and for the presence of the  $H-2^a$  allele. Backcross mice obtained using either ma-

TABLE III  
Immune Responses of Different Mouse Strains to Low Doses of Ovalbumin and Ovomuroid as Measured by Passive Cutaneous Anaphylactic Reactions. Relationship to Histocompatibility ( $H-2$ ) Type

$H-2$ type	Mouse strains	Responses to ovomucoid (1.0 $\mu$ g)			Responses to ovalbumin (0.1 $\mu$ g)		
		14th	28th	7th (Secondary)	14th	28th	7th (Secondary)
<i>a</i> <i>k</i>	A/J	10 (20)*	40 (40)	640 (640)	0 (0)	0 (0)	40 (40)
	AKR/J	10 (40)	40 (40)	640 (160)	0 (0)	0 (5)	80 (80)
	CBA/J	20 (40)	20 (40)	320 (160)	0 (0)	0 (0)	40 (80)
	C3H/HeJ	0 (0)	0 (0)	320 (80)	0 (0)	0 (0)	0 (0)
	B10.BR	10 (20)	40 (40)	640 (640)	0 (0)	0 (0)	40 (40)
<i>b</i>	C57BL/10J	0 (0)	0 (0)	0 (0)	10 (10)	5 (0)	320 (160)
	A.BY	0 (0)	0 (0)	0 (0)	40 (40)	80 (40)	320 (160)
	LP/J	0 (0)	0 (0)	5 (80)	5 (10)	40 (40)	160 (80)
<i>d</i>	DBA/2J	0 (0)	0 (0)	0 (0)	0 (0)	0 (5)	40 (160)
	B10.D2(new)	0 (0)	0 (0)	0 (0)	40 (5)	10 (5)	80 (20)
<i>q</i>	DBA/1J	0 (0)	0 (0)	10 (40)	160 (80)	160 (80)	1280 (640)
	SWR/J	0 (0)	0 (0)	5 (0)	80 (80)	80 (80)	1280 (320)
<i>a/b</i>	B6AF <sub>1</sub>	0 (0) ‡	0 (0)	80 (80)	5 (5)	20 (20)	80 (80)
		0 (0)	0 (0)	80 (80)	5 (40)	10 (10)	160 (160)
<i>a/q</i>	ARF <sub>1</sub>	0 (0)	0 (0)	160 (80)	80 (80)	160 (80)	1280 (160)
		0 (5)	20 (20)	160 (160)	80 (80)	160 (80)	1280 (160)
<i>a/d</i>	CAF <sub>1</sub>	5 (0)	20 (20)	160 (160)	5 (0)	10 (20)	160 (80)
		0 (0)	40 (20)	160 (160)	0 (0)	10 (10)	160 (80)
<i>k/d</i>	C3D2F <sub>1</sub>	0 (0)	20 (20)	160 (160)	0 (0)	0 (0)	40 (80)
		0 (5)	20 (20)	160 (160)	0 (0)	0 (0)	40 (80)
<i>b/d</i>	B6D2F <sub>1</sub>	0 (0)	0 (0)	0 (0)	80 (80)	160 (80)	1280 (160)
		0 (0)	0 (0)	0 (0)	80 (80)	160 (80)	1280 (160)

\* Both the IgG<sub>1</sub> titers and the IgE titers (in parentheses) are shown as the reciprocals of the highest dilutions of serum pools provoking threshold PCA reactions. For the parental strains the titers refer to serum pooled from the mice shown in Table I.

‡ For the F<sub>1</sub> hybrids, two distinct serum pools, from the first three and last three mice in each group shown in Table II were assayed independently.

ternal or paternal C57BL/6J parents segregated identically so the results were pooled. Small numbers of control parental and F<sub>1</sub> hybrid mice of both sexes were included in the experiments. The results are shown in Table V. Virtually all the A/HeJ mice, and well as the F<sub>1</sub> hybrids responded well to ovomucoid, whereas none of the C57BL/6J mice responded detectably. 17 male and 27

TABLE IV  
Correlation between Titers of Antibody Assayed by Antigen-Binding Methods and Passive Cutaneous Anaphylaxis with 2-Hr Sensitization Periods (IgG1)

Antigen-binding capacity	PCA titers		
	Low (0/1:20)	Moderate (1:40/1:160)	High (1:320 or higher)
Low (0-1.0)*	58‡	10	0
Moderate (1.1-10.0)	1	10	5
High (10.1 or higher)	0	1	14

\*  $\mu$ moles ( $\times 10^{-5}$ ) of antigen bound/ml of serum at the 35% point. Total number of comparisons: 99.

‡ Number of antisera.

TABLE V  
Linkage between Immune Responsiveness to Ovomuroid and the *H-2<sup>a</sup>* Allele in Mice from Backcross Generations

Mouse strain	Sex	Number of mice	<i>H-2<sup>a</sup></i> allele*	Anti-ovomuroid antibody titers		
				ABC‡	IgG1§	IgE
A/HeJ	♂	7	+	10;517;1;562; 262;600;490	80;160;0;160; 160;160;160	640;160;0;320; 160;640;320
C57BL/6J	♀	8	-	0;0;0;0;0;0;0	0;0;0;0;0;0;0	0;0;0;0;0;0;0
F <sub>1</sub> (AxC57)	♂	10	+	1;2;3;-;5;31; 32;1;31;8	40;80;40;5;40; 40;80;5;80;40	80;80;80;20;80; 160 10;80;160;160
F <sub>1</sub> (AxC57)	♀	7	+	195;410;147; 33;232;29;78	80;320;80;160; 160;40;80	80;160;320;320; 80;160;160
F <sub>1</sub> × C57	♂	17	7+	0;18;49;50;9; 42;4	0;5;80;80;5;80; 5	0;40;80;40;10; 160;40
			10-	0;0;0;0;0;0;0; 0;0	0;0;0;0;0;0;0; 0;0	0;0;0;0;0;0;0; 0;0
F <sub>1</sub> × C57	♀	27	14+	2;21;43;125;0; 57;12;38;9 129;22;2;12;218	80;0;10;80;0; 160;40;40 40;160;40;40; 80;80	80;5;20;40;10; 80;40;20;40 80;80;80;80;40
			13-	0;0;0;1;0;0;0;0; 0;0;0;0;0	0;0;0;0;0;0;0; 0;0;0;0;0	0;0;0;0;0;0;0; 0;0;0;0;0

All animals were immunized by two injections of 1.0  $\mu$ g of ovomucoid in 1 mg of Al(OH)<sub>3</sub>, intraperitoneally, given 4 wk apart, and bled 9 days after the second injection for antibody assays.

\* As measured by dextran hemagglutination with alloantisera specific for the *H-2<sup>a</sup>* allele.

‡ Expressed as  $\mu$ moles ( $\times 10^{-5}$ ) of ovomucoid bound/ml of serum at 35% point.

§ Expressed as reciprocal of PCA titers with 2-hr sensitization periods.

|| Expressed as reciprocal of PCA titers with 48-hr sensitization periods.



female mice from the backcross generation were tested. From the 17 males, seven were  $H-2^a$  positive, and six responded vigorously to OM; the 10 remaining mice were  $H-2^a$  negative and none responded to the immunization. From the 27 females, 14 were  $H-2^a$  positive and responded to OM, whereas 13 were  $H-2^a$  negative and did not respond to OM. These results are summarized in Table VI and demonstrate that immune responsiveness to low doses of OM is under control of a single dominant gene which is closely linked to the  $H-2^a$  allele.

*Responsiveness of  $H-2^h$  and  $H-2^i$  Mice to OM.*—A few experiments were made with  $H-2^h$  and  $H-2^i$  mice which are recombinants of  $H-2^a$  and  $H-2^b$  alleles; in  $H-2^h$  mice, the right-hand portion of the  $H-2$  region is like  $H-2^a$  and the left-hand portion is like  $H-2^b$ ; in  $H-2^i$  mice the situation is reversed (1, 19, 20). The responsiveness of  $H-2^h$  mice to low doses of OM was like  $H-2^a$ , whereas  $H-2^i$  mice were poor responders like  $H-2^b$ .

TABLE VI  
*Association between the  $H-2^a$  Allele and Immune Responsiveness to Ovomuroid in the Backcross Generations*

$H-2$ type	Responsiveness to OM	
	High responders	Low responders
$H-2^a$ positive	18	3
$H-2^a$ negative	0	23

Segregation of  $H-2^a$  according to 1:1 ratio:  $X^2 = 0.023$ ;  $0.95 < P < 0.99$ .

Segregation of responsiveness to OM according to 1:1 ratio:  $X^2 = 1.12$ ;  $0.20 < P < 0.30$ .

Nonindependence of segregation:  $X^2 = 29.9$ ;  $P < 0.001$ .

#### DISCUSSION

The present results demonstrate that high immune responsiveness to low doses of ovomucoid in the mouse is under control of a single dominant gene which is closely linked to the  $a$  and  $k$  alleles of the  $H-2$  locus, whereas high responsiveness to low doses of ovalbumin is associated with the  $b$ ,  $d$ , and  $q$  alleles.

Three points are worth discussing at the outset. The first point is that the gene action is concerned with the recognition of immunogenicity, and not with the synthesis of specific immunoglobulin types: once started, the immune response consisted of the production of a heterogeneous population of antibodies including globulins of different classes, e.g., IgG<sub>1</sub> and IgE (Table III). This is in agreement with previous findings on the genetic control of immune responsiveness (1-3, 6). The second point is that this control is exerted on responsiveness to low doses of immunogen; poorly responsive strains of mice may respond to immunization with higher doses of the same immunogen, as we actually observed when they were subsequently challenged with large doses of antigen (6, 13). The third point is that although the genes linked to the  $H-2$

locus control a major portion of the responsiveness of the strains in our system, there were variations which could not be ascribed to the presence of a particular *H-2* allele. Thus, among *H-2<sup>k</sup>* mice, the AKR/J strain responded better to both ovomucoid and ovalbumin (although poorly to the latter) than the other *H-2<sup>k</sup>* strains. As the animals used were all young adult females, factors such as sex (21, 22) and age, which are known to be important for immune responsiveness, cannot explain these differences.

Concerning the fact that genetic control of immune responsiveness is best observed with low doses of immunogen, it must be stressed that most of the immunogenic stimuli met by an organism during its normal life are probably of small magnitude. Thus, genetic controls, such as the one described should be fully operative under physiological conditions.

Responsiveness to low doses of immunogen may depend on one or more of the following factors. Responder animals may have a higher number of precursor lymphoid cells available for the primary immune response, as demonstrated recently for a synthetic polymer (23, 24), or they may have more efficient mechanisms for concentrating specific immunogen molecules and exposing them to precursor cells; the presence of 'natural' antibodies might represent one of these mechanisms (25). High doses of immunogen might override the genetic control by bringing into play receptors with low binding affinities for the immunogen. Alternatively, poorly responsive strains to low doses of immunogen might make strong immune responses initially directed against antigenic determinants only adequately represented in high doses of immunogen. Several experiments have demonstrated that animals with different genetic backgrounds may respond to different portions of the same immunogen molecule (26-29). It has also been shown that recognition of one portion of an immunogenic molecule, or complex of molecules, may lead to the formation of antibodies directed against other portions of the immunogen (30-34).

The linkage between 'specific immune response genes' and histocompatibility loci has now been observed in too many diverse systems to be merely coincidental. These genes might be linked to *H* loci but might not necessarily code for products detectable on the cell membrane as alloantigenic determinants (specificities). However, it seems highly probable that the gene products are expressed on the cell membrane, or some other site readily accessible to immunogen molecules. Alternatively, specific immune response genes might just represent pleiotropic effects of *H* loci, e.g., histocompatibility antigens might themselves form receptors for immunogens on the membrane of antigen-sensitive cells, or might sterically interfere with, or enhance, the immunogen-cell interaction. However, an altogether different explanation for these phenomena has been proposed: it has been suggested that self-antigens, such as histocompatibility antigens, might be involved in the generation of antibody diversity (35).

Although interstrain differences in immune responsiveness to OM and OA

were observed within the same *H-2* allelic type, broadly speaking, *H-2<sup>a</sup>* and *H-2<sup>k</sup>* mice on one hand, and *H-2<sup>b</sup>*, *H-2<sup>d</sup>*, and *H-2<sup>q</sup>* mice on the other hand, behaved similarly. The *H-2* locus is a complex genetic unit coding for many different alloantigenic specificities, each allele being represented by a particular collection of them. Of the known specificities, only *H-2.1* and *H-2.25* are found to be present in *a* and *k* genotypes and missing in *b*, *d*, and *q* genotypes (19). These specificities are both coded by genes which map on the right-hand portion of the *H-2* region (20). The *Ir-1* gene, which controls the responsiveness of mice to a series of branched, multichain polypeptide antigens (1-3), maps adjacently to specificity *H-2.1* as indicated by the responsiveness of recombinant strains. The pattern of responsiveness of *H-2<sup>k</sup>* and *H-2<sup>i</sup>* mice to OM is consistent with the idea that the gene controlling this response also maps in this region. However, the distribution of *H-2* genotypes responsive to OM is similar but not identical to that of responders to the polymer (H, G)-A-L; likewise, the distribution of responders to OA is similar but not identical to that of responders to (T,G)-A-L (1, 3, 6, 7). Furthermore, although specificities *H-2.1* and *H-2.25* might be considered genetic markers for the ability to respond to low doses of OM, the present studies failed to identify any markers for the ability to respond to low doses of OA. The absence of *H-2.1* and *H-2.25* cannot be used as such a marker because F<sub>1</sub> hybrids, which do express these specificities, respond well to OA (Table II), i.e., responsiveness is a dominant trait. Other systems linked to *H* loci have been described, however, in which responsiveness is inherited as a recessive trait. In these systems, nonresponsiveness was proven not to be because of cross-tolerance to self-antigens (8, 9, 36).

The immune responsiveness of guinea pigs to polymers of L-glutamic acid, L-alanine, and L-tyrosine (GA, GT, and GAT) has been recently shown (37) to be under control of dominant genes which are closely linked, but not identical to the PLL (poly-L-lysine) gene. Strain 2 guinea pigs responded to GA and to the GA portion of GAT; strain 13 guinea pigs responded to GT and to the GT portion of GAT, but not to GA. In noninbred guinea pigs, responsiveness to GA tended to exclude responsiveness to GT, indicating allelism or pseudo-allelism between the genes controlling these immune responses. These findings are very similar to the presently described responsiveness of mice to limiting doses of protein antigens.

#### SUMMARY

Immune responsiveness of inbred mice to low doses of ovalbumin or ovomucoid is under control of single dominant genes closely linked to alleles of the *H-2* locus. High responsiveness to ovomucoid is linked with the *H-2<sup>a</sup>* and *H-2<sup>k</sup>* alleles, and to ovalbumin with the *H-2<sup>b</sup>*, *H-2<sup>d</sup>*, and *H-2<sup>q</sup>* alleles.

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## REFERENCES

1. McDevitt, H. O., and B. Benacerraf. 1969. Genetic control of specific immune responses. *Advan. Immunol.* **11**:31.
2. McDevitt, H. O., and M. L. Tyan. 1968. Genetic control of the antibody response in inbred mice. Transfer of response by spleen cells and linkage to the major histocompatibility (*H-2*) locus. *J. Exp. Med.* **128**:1.
3. McDevitt, H. O., and A. Chinitz. 1969. Genetic control of the antibody response: relationship between immune response and histocompatibility (*H-2*) type. *Science (Washington)*. **163**:1207.
4. Merryman, C., and P. H. Maurer. 1971. Genetic control of immune response to synthetic polypeptides in mice. *Fed. Proc.* **30**:470.
5. Skarova, B., and I. Riha. 1969. Genetic control of the immune response. I. Differences in immune responses in inbred strains of mice to *p*-aminobenzoic acid and sulphanilic acid. *Folia Microbiol.* **14**:364.
6. Vaz, N. M., and B. B. Levine. 1970. Immune responsiveness of inbred mice to repeated low doses of antigen: relationship to histocompatibility (*H-2*) type. *Science (Washington)*. **168**:852.
7. Vaz, N. M., E. M. Vaz, and B. B. Levine. 1970. Relationship between histocompatibility (*H-2*) genotype and immune responsiveness to low doses of ovalbumin in the mouse. *J. Immunol.* **104**:572.
8. Rathbun, W. E., and W. H. Hildeman. 1970. Genetic control of the antibody response to simple haptens in congenic strains of mice. *J. Immunol.* **105**:98.
9. Gasser, D. L. 1969. Genetic control of the immune response in mice. I. Segregation data and localization to the fifth linkage group of a gene affecting antibody production. *J. Immunol.* **103**:66.
10. Gasser, D. L. 1970. Genetic control of the immune response in mice. II. A two-way selection experiment for specific immunologic competence. *J. Immunol.* **105**:908.
11. Gasser, D. L., and W. K. Silvers. 1971. Genetic control of the immune response in mice. III. An association between *H-2* type and reactions to H-Y. *J. Immunol.* **106**:875.
12. Ellman, L., I. Green, W. J. Martin, and B. Benacerraf. 1970. Linkage between the poly-L-lysine gene and the locus controlling the major histocompatibility antigens in strain 2 guinea pigs. *Proc. Nat. Acad. Sci. U.S.A.* **66**:322.
13. Green, I., J. K. Inman, and B. Benacerraf. 1970. Genetic control of the immune response of guinea pigs to limiting doses of bovine serum albumin. Relationship to the poly-L-lysine gene. *Proc. Nat. Acad. Sci. U.S.A.* **66**:1267.
14. Kabat, E. A., and M. M. Mayer. 1961. Kjeldahl nitrogen determination. *In* Experimental Immunochemistry. Charles C Thomas, Publisher, Springfield, Ill. 476.
15. Levine, B. B., and N. M. Vaz. 1970. Effect of combinations of inbred strain, antigen and antigen dose on immune responsiveness and reagin production in the mouse. A potential mouse model for immune aspects of human atopic allergy. *Int. Arch. Allergy Appl. Immunol.* **39**:156.
16. Mitchison, N. A. 1968. The dosage requirements for immunological paralysis by soluble proteins. *Immunology.* **15**:509.

17. McFarlane, A. S. 1958. Iodine monochloride iodination of proteins. *Nature (London)*. **182**:53.
18. Stimpfling, J. H. 1966. Methods for detection of hemagglutinins in mouse isoantisera. *Methods Med. Res.* **10**:22.
19. Snell, G. D., and J. H. Stimpfling. 1966. Genetics of tissue transplantation. *In* Biology of the Laboratory Mouse. E. L. Green, editor. McGraw Hill Book Company, New York.
20. Schreffler, D. C., and J. Klein. 1970. Genetic organization and gene action of mouse H-2 region. *Transplant. Proc.* **2**:5.
21. Hildeman, W. H., and N. Cohen. 1967. *In* Histocompatibility Testing. E. S. Curton, P. L. Mattiuz, and R. M. Tosi, editors. E. Munksgaard, Copenhagen. 13.
22. Terres, G., S. L. Morrison, and G. S. Habicht. 1968. A quantitative difference in the immune response between male and female mice. *Proc. Soc. Exp. Biol. Med.* **127**:664.
23. Mozes, E., G. M. Shearer, and M. Sela. 1970. Cellular basis of the genetic control of immune responses to synthetic polypeptides. I. Differences in frequency of splenic precursor cells specific for a synthetic polypeptide derived from multichain polyproline, ([T, G]-Pro-L) in high and low responder inbred mouse strains. *J. Exp. Med.* **132**:613.
24. Shearer, G. M., E. Mozes, and M. Sela. 1971. Cellular basis of the genetic control of immune responses to synthetic polypeptides. II. Frequency of immunocompetent precursors specific for two distinct regions within ([Phe, G]-Pro-L), a synthetic polypeptide derived from multichain polyproline, in inbred mouse strains. *J. Exp. Med.* **133**:216.
25. Jerne, N. K. 1960. Immunological speculations. *Annu. Rev. Microbiol.* **14**:341.
26. Arquilla, E. R., and J. Finn. 1963. Genetic differences in antibody production to determinant groups on insulin. *Science (Washington)*. **142**:400.
27. Mozes, E., H. O. McDevitt, J. C. Jaton, and M. Sela. 1969. The nature of the antigenic determinant in a genetic control of the antibody response. *J. Exp. Med.* **130**:493.
28. Mozes, E., H. O. McDevitt, J. C. Jaton, and M. Sela. 1969. The genetic control of antibody specificity. *J. Exp. Med.* **130**:1263.
29. Mozes, E., E. Maron, R. Arnon, and M. Sela. 1971. Strain dependent differences in the specificity of antibody responses toward lysozyme. *J. Immunol.* **106**:862.
30. Green, I., W. E. Paul, and B. Benacerraf. 1966. The behavior of hapten-poly-L-lysine conjugates as complete antigens in genetic responders and as haptens in nonresponder guinea pigs. *J. Exp. Med.* **123**:859.
31. McBride, R. A., and L. W. Schierman. 1970. Hapten-carrier relationships of isoantigens. A model for immunological maturation based on the conversion of haptens to carriers by antibodies. *J. Exp. Med.* **131**:377.
32. Rajewsky, K., and E. Rotlander. 1967. Tolerance specificity and the immune response to lactic dehydrogenase isoenzymes. *Cold Spring Harbor Symp. Quant. Biol.* **32**:547.
33. Stimpfling, J. H., and C. R. McBroom. 1971. The effect of H-2 on the humoral antibody response to a non-H-2 blood group antigen. *Transplantation*. **11**:87.

34. Pincus, C. S., M. E. Lamm, and V. Nussenzweig. 1971. Regulation of the immune response: suppressive and enhancing effect of passively administered antibody. *J. Exp. Med.* **133**:987.
35. Jerne, N. K. 1970. Generation of antibody diversity and self-tolerance: a new theory. *In* Immune Surveillance. R. T. Smith and M. Landy, editors. Academic Press, Inc., New York. 343.
36. Bechtol, K. B., T. G. Wegman, B. W. Chesebro, L. A. Herzenberg, and H. O. McDevitt. 1971. The antibody response to (T,G)-A-L in tetraparental mice. *Fed. Proc.* **30**:469.
37. Bluestein, H. G., I. Green, and B. Benacerraf. 1971. Genetic control of immune responses of guinea pigs to GA, GT and GAT. *Fed. Proc.* **30**:469.