GENETIC CONTROL OF THE IMMUNE RESPONSE

MAPPING OF THE *IR-1* LOCUS*

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(Received for publication 7 February 1972)

Genetic control of the immune response to specific antigens or specific antigenic determinants has now been demonstrated for a wide variety of antigens in several different animal species (1). The ability of inbred strains of mice to produce antibody against branched, multichain, synthetic polypeptide antigens is a quantitative, autosomal dominant trait which has been shown to be linked to the major histocompatibility (*H-2*)[1] locus in the IXth mouse linkage group (2). During the past 3 yr, specific immune response genes in the mouse, the guinea pig, and the rat, controlling graft rejection, delayed sensitivity, or antibody production to more than 20 different antigens, have all been shown to be linked to the genes controlling the species' major histocompatibility antigens (3). The nature, significance, and extent of this relationship between specific immune response genes and histocompatibility antigens is not yet known. One important approach to this problem is to determine the genetic relationship between murine-specific immune response genes and histocompatibility antigens by attempting to map the precise location of the immune response genes in the IXth mouse linkage group, with particular reference to the chromosome region controlling the histocompatibility (*H-2*) antigenic specificities.

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* These studies were supported by grants from the US Public Health Service: AI-07757, AI-10032, GM-15419, AI-06525, GM-18314, CA-01329 and from the American Cancer Society, California Division, No. 505.

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§ Recipient of Career Development Award HE-24980 from US Public Health Service.

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[1] *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; *H-2*, histocompatibility-2; *Ir-1*, immune response-1; PBS, phosphate-buffered isotonic saline.

Sufficient knowledge of the IXth mouse linkage group (4), and of the genetic fine structure of the *H-2* region (5), is available to make such an attempt feasible. Fig. 1 is a schematic diagram of the IXth mouse linkage group, with particular emphasis on the fine structure of the *H-2* complex (5). The *T-t* complex maps closest to the centromere and consists of one dominant (*T*) and several recessive factors (*t* alleles) with a variety of effects on the development of the notochord, neural tube, and spinal column (6). The dominant factor *T* (brachyury) is apparently a point mutation which in the *T*/ + heterozygote leads to shortening of the tail. The homozygous *T*/*T* state is lethal. The *tf* (tufted) locus controls hair growth pattern (7). The *tf*/*tf* homozygote has an abnormal hair growth pattern with serial waves of hair loss proceeding from head to tail, beginning at about the 7–8th wk of age. The *H-2* complex is a large chromosomal region consisting of at least two loci, *H-2K* and *H-2D*, governing the antigenic specificities of the major histocompatibility antigens expressed on the cell surface. Crossing over has been demonstrated between *H-2K* and *H-2D* at a rate of about 0.5%. These two loci of the *H-2* complex are separated by the *Ss-Slp* complex which controls a serum alpha globulin detectable by a rabbit anti-mouse antiserum (*s*erologically detected *s*erum protein), as well as an allotypic variation in the *Ss* protein which is expressed only in males and is therefore designated as a *s*ex-*l*imited *p*rotein (Slp) (8). The *Ss-Slp* loci are closely linked, genetically indistinguishable loci, and are useful markers for localization of crossovers within the *H-2* complex. There are two alleles of *Ss*, namely Ss^l and Ss^h, for low and high levels of the *Ss* protein, as well as Slp^a and Slp^o which determine the presence or absence of the allotypic variant of the *Ss* protein. Since the known *H-2* alleles carried by the various inbred mouse strains can be typed with respect to their *Ss* and *Slp* type, as well as for the private *H-2* antigenic specificities controlled by the *H-2K* and *H-2D* loci of the *H-2* complex, crossovers between the *H-2K* and *H-2D* antigenic specificities can be localized with respect to the *Ss-Slp* complex. The *Ir-1* locus (*i*mmune *r*esponse to branched, multichain, synthetic polypeptide antigens) is positioned in this schematic diagram on the basis of the results to be described in this paper.

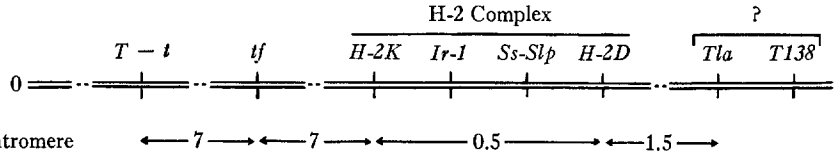

FIG. 1. From: Klein, J., and D. C. Shreffler. 1971. The *H-2* model for the major histocompatibility systems. *Transplant. Rev.* **6**:3.

The present study combines two methods of genetic mapping: (*a*) the determination of the immune response to branched, synthetic polypeptide antigens given by mouse strains bearing recombinant *H-2* chromosomes derived from intra-*H-2* crossovers between chromosomes of known *H-2* type and known immune response gene type, and (*b*) a four-point linkage cross utilizing *H-2*, *tf* (tufted hair pattern), *T* (brachyury), and *Ir-1* (immune response to branched, multichain, synthetic polypeptide antigens). The results show that *Ir-1* maps within the *H-2* complex lying between the *H-2K* locus and the *Ss-Slp* loci.

Materials and Methods

The inbred strains, congenic resistant lines, and strains which are not inbred but are homozygous for known recombinant *H-2* chromosomes are listed in Table I.

The antigens used in this study were: poly-L-(tyrosine, glutamic acid)-poly-DL-alanine--poly-L-lysine [(T,G)-A--L], preparation No. 509 (9); poly-L-(histidine, glutamic acid)-poly-DL-alanine--poly-L-lysine [(H,G)-A--L], preparation Nos. 905 and 1201 (10, 11); poly-L-(phenylalanine, glutamic acid)-poly-DL-alanine--poly-L-lysine [(Phe,G)-A--L], preparation No. 223 (11).

TABLE I

Strains Employed

Strain	Synonym	*H-2* Allele	Recombinant *H-2* derivation*
A/He	A	a	(K^kD^d)
C3H.SW	CSW	b	—
C57BL/6	C57	b	—
BALB/c	—	d	—
C3H/He	C3H	k	—
C3H/DiSn	C3H	k	—
DBA/1	D1	q	—
A.SW	—	s	—
B10.A(1R)	—	h	(K^aD^b)
B10.A(2R)	—	h	(K^aD^b)
B10.A(3R)	—	i	(K^bD^a)
B10.A(4R)	—	h	(K^aD^b)
B10.A(5R)	—	i	(K^bD^a)
H-2^{oh}	(not inbred) oh	o	(K^dD^k)
H-2^{ol}	(not inbred) ol	o	(K^dD^k)
H-2^{al}	(not inbred) al	a	(K^kD^d)
H-2^{tl}	(not inbred) tl	t	(K^sD^{al})
H-2th	(not inbred) th	t	(K^sD^a)
AQR	(not inbred)	y	(K^qD^a)
Linkage stock (Dr. M. Green)	(not inbred)	q	—
T(2:9)138Ca	T138	q	—

* The notation used here follows the recent relocation of the centromere in the IXth mouse linkage group (see references 16–18).

Mice were immunized with 10 μg of (T,G)-A--L 509; 100 μg of (H,G)-A--L 1201; 10 μg of (H,G)-A--L 905; or 100 μg of (Phe,G)-A--L 223 emulsified in complete Freund's adjuvant (CFA) and injected in the hind footpads as described previously (9–11). A second injection of the same amount of the same antigen dissolved in phosphate-buffered isotonic saline, pH 7.4 (PBS), was given in the hind footpads 3 wk later. Serum for antibody titrations was obtained by tail vein bleeding 10 days after the secondary stimulus (9–11).

Antibody response was measured by an antigen-binding assay (2) in which (T,G)-A--L labeled with ^{125}I (2), or (H,G)-A--L or (Phe,G)-A--L labeled with acetic anhydride-^3H (11) was mixed with appropriate dilutions of mouse antisera. After incubation, labeled antigen bound to antibody was precipitated by the addition of appropriate dilutions of a polyvalent

rabbit anti-mouse γ-globulin (2). Results were expressed as per cent of antigen bound at a specific antiserum dilution.

Mice were maintained in standard plastic cages, on wood shavings, and were fed Purina laboratory chow or breeder chow (Ralston Purina Co., Inc., St. Louis, Mo.), as indicated. In the four-point linkage test, initial mating pairs were set up between mice of the A.SW strain and mice carrying a special set of markers in the IXth mouse linkage group. These latter mice were kindly supplied by Dr. Margaret C. Green, The Jackson Laboratory, Bar Harbor, Maine. The A.SW strain is wild type (+) for the *T* (brachyury) and *tf* (tufted hair pattern) loci, carries the *H-2*^{s} allele, and is a low responder to (T,G)-A--L, (H,G)-A--L, and (Phe,G)-A--L. The A.SW genotype is thus + + *H-2*^{s} *Ir-1*$^{(T,G)\text{-low, }(H,G)\text{-low, }(Phe,G)\text{-low}}$/+ + *H-2*^{s} *Ir-1*$^{(T,G)\text{-low, }(H,G)\text{-low, }(Phe,G)\text{-low}}$. The linkage testing stock mice supplied by Dr. Green were of the following genotype: *T tf H-2*^{q} *Ir-1*$^{(T,G)\text{-low, }(H,G)\text{-low, }(Phe,G)\text{-high}}$/+ *tf H-2*^{q} *Ir-1*$^{(T,G)\text{-low, }(H,G)\text{-low, }(Phe,G)\text{-high}}$.

In such a cross, 50% of the offspring will receive the *T* allele and will be short-tailed, while 50% will be wild type at the *T* locus. Short-tailed (*T*) F_1 animals were used for the backcross

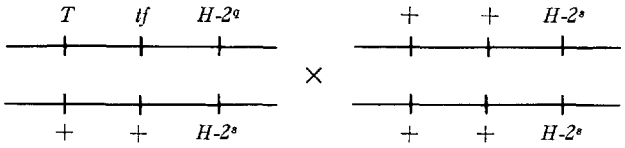

FIG. 2. *Ir-1*^{s} mapping cross. Modified from: McDevitt, H. O., D. C. Shreffler, G. D. Snell, and J. H. Stimpfling. 1971. *In* Immunogenetics of the *H-2* System, Symposium, Liblice-Praha, 1970. S. Karger, AG, Basel.

H-2^q = high response to (Phe,G)-A--L
H-2^s = low response to (Phe,G)-A--L

to A.SW. The genotypes used in the backcross are shown in Fig. 2. The locallization of the *Ir-1* locus is not shown in this diagram, since this is the locus to be mapped. The *Ir-1* genotype of the input parental chromosomes is given above and in Fig. 2. In brief, the *H-2*^{q} allele is linked to high response to (Phe,G)-A--L, while the *H-2*^{s} allele is linked to low response to (Phe,G)-A--L.

All parental, F_1, and A.SW mice, as well as their offspring, were ear-tagged as soon as this was feasible. The offspring from this backcross were scored by inspection for the presence or absence of the *T* allele. This trait has approximately a 90% penetrance (6), so that about 10% of the animals bearing the *T* allele would be missed. Offspring were scored for the *tf* allele by crossing them to the original linkage stock, since *tf* is a recessive gene. In the event that no tufted offspring were obtained, crossing to the original linkage stock was continued wherever possible until at least eight offspring had been obtained. If none of eight offspring exhibited the tufted hair pattern by 12 wk of age, then the animal under test was scored +/+ at the *tf* locus. *H-2* typing was carried out by hemagglutination, using the polyvinylpyrrolidone method (12). The presence of antigens specified by the *H-2*^{q} chromosome was detected by the following antisera:

(*a*) AS-348D (B10.A[5R] × LP.R III F_1) anti-DBA/1
Principal specificities: 17 and 30;
(*b*) C-13 (B10.BR × 129)F_1 anti-B10.AKM
Principal specificities: 13 and 30;

(*c*) AS-411B (A.SW × DBA/2)F_1 anti-DBA/1
Principal specificities: 25 and 30;
(*d*) AS-360 SJL anti-DBA/1
Principal specificities: 25 and 30.

The presence of *H-2* antigens specified by the *H-2*^{s} allele were detected by the following antiserum:

AS-338D (B10.Y × WB.Re)F_1 anti-A.SW
Principal specificities: 1, 3, 19.

Scoring for *Ir-1*$^{(Phe,G)\text{-}low}$ or *Ir-1*$^{(Phe,G)\text{-}high}$ was done by immunizing mice with (Phe,G)-A--L in CFA, followed by a booster injection of (Phe,G)-A--L in PBS, as described above. Antibody response to (Phe,G)-A--L was determined as described above.

As indicated in the Results section, all animals which appeared to be recombinants between *H-2* and *Ir-1* were subjected to progeny testing. Those animals which, on progeny testing, appeared to be true recombinants between *H-2* and *Ir-1* were subjected to further analysis of their *H-2* type by serologic testing (13, 14) and F_1 skin graft testing (15). The antisera used for serologic testing of *H-2* type, and the strains used for the F_1 skin graft test are given in the Results section.

RESULTS

Localization of the Ir-1 Locus by Determination of the Ir-1 Phenotype of Mouse Strains Bearing Recombinant H-2 Alleles.—

Recombination between the *H-2K* and *H-2D* loci of the *H-2* complex occurs with sufficient frequency (about 0.5%) to be detected in relatively small numbers of potential recombinant mice (on the order of 100–1000 animals). By appropriate crosses between inbred mouse strains of known *H-2* type, F_1 heterozygotes can be produced and backcrossed to either parental strain. In the backcross population, one-half of the animals will be heterozygous at the *H-2* complex, identical to the F_1 parent in the backcross. The offspring of such an F_1 × parent cross can then be tested for the presence or absence of *H-2* antigenic specificities controlled by the *H-2K* and *H-2D* loci of the *H-2* complex. For example, an *H-2*^{a}/*H-2*^{b} heterozygote is crossed with an *H-2*^{b}/*H-2*^{b} homozygote. Offspring are tested serologically for possession of the *H-2K* and *H-2D* specificities of the *H-2*^{a} chromosome. Animals which lack either the *H-2K*^{a} or *H-2D*^{a} specificities are then subjected to progeny testing, serologic analysis, and are tested for their ability to absorb in vivo antisera of known *H-2* antigenic specificity. When a proven recombinant is detected, the recombinant *H-2* chromosome can then be put on a known inbred mouse strain background by serial backcrossing, followed by an intercross and selection of the animals that are homozygous for the recombinant *H-2* chromosome.

In the present study, inbred and partially inbred mouse strains homozygous for known recombinant *H-2* chromosomes were utilized in an attempt to localize the *Ir-1* locus with respect to the *H-2K* and *H-2D* loci. For example, all mice of the *H-2*^{a}/*H-2*^{a} genotype are low responders to (T,G)-A--L and high responders to (H,G)-A--L, while all mice of the *H-2*^{b}/*H-2*^{b} genotype are high responders to (T,G)-A--L and low responders to (H,G)-A--L. By immunizing mice homozygous for recombinant *H-2* chromosomes derived from a known crossover event between *H-2*^{a} and *H-2*^{b}, we can expect to determine whether the *Ir-1* phenotype is identical with that of the donor of the *H-2K* or *H-2D* locus of the recombinant *H-2* chromosome. It must be emphasized that such

studies do not necessarily give a precise localization of the *Ir-1* locus, but would simply indicate whether the *Ir-1* locus is in the interval between *H-2K* and the centromere, or in the interval between *H-2D* and the telomere (the noncentromeric end of the chromosome).[2]

Thus, the initial aim of determining the *Ir-1* genotype of the known recombinant *H-2* chromosomes was simply to localize *Ir-1* in either the centromeric or noncentromeric part of the IXth mouse linkage group, using the two loci of the *H-2* complex as the division point.

The availability of the *Ss-Slp* genetic markers permits localization of a known crossover event between the *H-2K* and *H-2D* loci of the *H-2* complex, so that a crossover can be shown to have occurred between the *H-2K* locus and the *Ss* locus, or between the *Ss* locus and the *H-2D* locus.

In utilizing recombinant *H-2* chromosomes to localize the *Ir-1* locus in the IXth mouse linkage group, a number of assumptions have to be made. The first is that the event giving rise to the recombinant *H-2* chromosome is a single crossover, and that crossing over did not occur elsewhere in the IXth mouse linkage group. The second assumption is that crossing over is preceded by homologous pairing and does not involve either duplication of certain segments of the chromosome, or deletion of segments of one of the input chromosomes. The third assumption is that the *Ir-1* locus controlling immune response to (T,G)-A--L, (H,G)-A--L, and (Phe,G)-A--L is either a single locus or three very closely linked loci, and that the crossover event giving rise to the recombinant *H-2* chromosome did not occur at or between these closely linked loci. These assumptions are implicit in using the recombinant *H-2* chromosomes to localize any genetic trait in the IXth mouse linkage group, and while some of them can be verified, as will be seen below, others must remain assumptions which, although likely to be correct, are extremely difficult to prove.

The *Ir-1* phenotype was determined for 11 recombinant *H-2* chromosomes derived from crossovers between the *H-2K* and *H-2D* loci of known *H-2* chromosomes with known *Ir-1* genotype. Five of these *H-2* recombinants were derived from crossovers between $H\text{-}2^a$ and $H\text{-}2^b$ by Stimpfling (19, 20). Three of these recombinant *H-2* alleles were derived from crossovers between $H\text{-}2^d$ and $H\text{-}2^k$ (21). One each of the recombinant *H-2* chromosomes derived from: (*a*) a crossover between $H\text{-}2^a$ and $H\text{-}2^s$ (20); (*b*) a crossover between an artificially pro-

[2] The map positions for the *H-2K* and *H-2D* loci have been reversed from those referred to in previous publications, for example, see reference 3. This is due to a new finding (16-18) which places the centromere at the *H-2K* end of the *H-2* complex. In standard genetic practice, genetic markers are ordered from left to right, beginning with the centromere. Therefore, the map order of the IXth mouse linkage group is that given in Fig. 1. While this rearrangement is necessary to conform to standard practices, it may lead to confusion, since in earlier references to the localization of *Ir-1* with respect to the *H-2* complex, the *H-2K* region was designated as the *right*-hand, and the *H-2D* region as the *left*-hand part of the *H-2* complex. Throughout this paper, the new convention has been followed.

duced (by crossover) $H\text{-}2^a$ ($H\text{-}2^{a-Ss^l}$, or $H\text{-}2^{a^l}$) and $H\text{-}2^s$ (21); and (*c*) a crossover between $H\text{-}2^a$ and $H\text{-}2^q$ (15).

Table II gives the detailed results of studies of the immune response of strains bearing *H-2* chromosomes recombinant between $H\text{-}2^a$ and $H\text{-}2^b$. The

TABLE II

Immune Response of Strains Bearing H-2 Chromosomes Recombinant Between $H\text{-}2^a$ and $H\text{-}2^b$

Strain	*H-2* Type	Crossover diagram*	Immunizing antigen	No. of mice	% Antigen bound		Serum dilution‡	Antigen used for titration§
					Range	Average		
A	a	—	(T,G)-A--L 509	9	5–15	10	1/500	509
A	a	—	(H,G)-A--L 1201	8	61–83	77	1/10	1201
A	a	—	(Phe,G)-A--L 223	10	73–76	75	1/10	223
C3H.SW	b	—	(T,G)-A--L 509	20	46–84	71	1/500	509
C3H.SW	b	—	(H,G)-A--L 1201	8	0–13	<5	1/10	1201
C3H.SW	b	—	(Phe,G)-A--L 223	6	70–74	73	1/10	223
C57BL/10	b	—	(T,G)-A--L 509	9	21–64	42	1/500	509
C57BL/10	b	—	(H,G)-A--L 905	10	0–19	<5	1/10	905
C57BL/10	b	—	(Phe,G)-A--L 223	10	67–70	69	1/10	223
B10.A (1R)	h	K^a Ss^a | D^b	(T,G)-A--L 509	11	0	0	1/500	509
B10.A (2R)	h	K^a Ss^a | D^b	(T,G)-A--L 509	13	0	0	1/500	509
B10.A (3R)	i	K^b | Ss^a D^a	(T,G)-A--L 509	13	19–75	46	1/500	509
B10.A (4R)	h	K^a | Ss^b D^b	(T,G)-A--L 509	9	<5–22	5	1/500	509
B10.A (4R)	h	K^a | Ss^b D^b	(H,G)-A--L 905	9	68–74	71	1/10	905
B10.A (4R)	h	K^a | Ss^b D^b	(Phe,G)-A--L 223	6	42–58	53	1/500	509‖
B10.A (5R)	i	K^b | Ss^a D^a	(T,G)-A--L 509	9	32–57	43	1/500	509
B10.A (5R)	i	K^b | Ss^a D^a	(H,G)-A--L 905	5	0–20	6	1/10	905

* The notation used here follows the recent relocation of the centromere in the IXth mouse linkage group (references 16–18).

‡ Dilutions for (H, G)-A--L 905 and 1201 and (Phe,G)-A--L 223 were 1/10 to give equimolar amounts of antigen and antiserum with respect to the assay for (T,G)-A--L 509 (1/500 serum dilution).

§ (H,G)-A--L 905 and 1201 gave equal % antigen-bound values in this assay. In addition, both elicited identical immune responses in high-responder and low-responder strains (McDevitt, H. O., and E. Mozes. Unpublished data).

‖ In a few instances, the extensive cross-reactions between antisera to these antigens were utilized to titer antisera to (H,G)-A--L or (Phe,G)-A--L after the supply of 905-^{3}H, 1201-^{3}H, and 223-^{3}H were exhausted (reference 23).

immune response characteristic of all strains bearing the two parental chromosomes, $H\text{-}2^a$ and $H\text{-}2^b$, is shown in the top part of the table. The remainder of the table lists the five *H-2* recombinants derived from crossovers between $H\text{-}2^a$ and $H\text{-}2^b$ (19, 20). In the schematic crossover diagram for each of the *H-2* recombinant alleles, the donors of the *H-2K* and *H-2D* loci are indicated by *a* or *b*. The *Ss* locus is positioned between the *H-2K* and *H-2D* loci. The position of the crossover giving rise to the recombinant *H-2* allele is indicated

by the dotted line, and was deduced from determining the *Slp* genotypes of the *H-2* recombinants (8). Comparison of the immune response to (T,G)-A--L, (H,G)-A--L, and (Phe,G)-A--L, of strains bearing the parental $H\text{-}2^a$ and $H\text{-}2^b$ chromosomes, with the immune response of strains bearing the *H-2* recombinants ($H\text{-}2^h$ and $H\text{-}2^i$), shows that in all cases the strains bearing the *H-2* recombinants respond to these antigens in the same way as do the donors of the *H-2K* locus, or centromeric part of the recombinant *H-2* complex. Therefore, on the basis of these results, and the assumptions listed above, the *Ir-1* locus is localized to the centromeric or *H-2K* region of the *H-2* complex.

TABLE III

Immune Response of Strains Bearing H-2 Chromosomes Recombinant Between $H\text{-}2^d$ and $H\text{-}2^k$

Strain	*H-2* type	Crossover diagram*	Immunizing antigen	No. of mice	% Antigen bound		Serum dilution‡	Antigen used for titration§
					Range	Average		
BALB/c	d	—	(T,G)-A--L 509	8	0–55	28	1/500	509
BALB/c	d	—	(H,G)-A--L 1201	10	15–63	42	1/10	1201
BALB/c	d	—	(Phe,G)-A--L 223	9	68–75	72	1/10	223
C3H	k	—	(T,G)-A--L 509	10	8–46	19	1/500	509
C3H	k	—	(H,G)-A--L 905	8	61–82	71	1/10	1201§
C3H	k	—	(Phe,G)-A--L 223	10	72–75	74	1/10	223
$H\text{-}2^{o\text{-}Ss^l}$	o^l	K^d | Ss^k D^k	(H,G)-A--L 905	25	<5–66	32	1/10	905
$H\text{-}2^{o\text{-}Ss^h}$	o^h	K^d Ss^d | D^k	(H,G)-A--L 905	14	6–58	32	1/10	905
$H\text{-}2^{o\text{-}Ss^h}$	o^h	K^d Ss^d | D^k	(H,G)-A--L 905	12	0–43	20	1/10	905
$H\text{-}2^{a\text{-}Ss^l}$	a^l	K^k Ss^k | D^d	(H,G)-A--L 905	13	67–76	72	1/10	905

* The notation used here follows the recent relocation of the centromere in the IXth mouse linkage group (references 16–18).

‡ Dilutions for (H,G)-A--L 905 and 1201 and (Phe,G)-A--L 223 were 1/10 to give equimolar amounts of antigen and antiserum with respect to the assay for (T,G)-A--L 509 (1/500 serum dilution).

§ (H, G)-A--L 905 and 1201 gave equal % antigen-bound values in this assay. In addition, both elicited identical immune responses in high-responder and low-responder strains (McDevitt, H. O., and E. Mozes. Unpublished data).

The immune response of strains bearing *H-2* chromosomes recombinant between $H\text{-}2^d$ and $H\text{-}2^k$ are shown in Table III. As in Table II, the top part of the table lists the details of the immune response characteristic of all $H\text{-}2^d$ and $H\text{-}2^k$ strains of mice. The bottom part of the table lists the *H-2* recombinants borne by the strains tested, a schematic diagram of the crossover, similar to that in Table II, and the immune response of these strains to the antigens tested. Once again, comparison of the immune response of the parental $H\text{-}2^d$ and $H\text{-}2^k$ strains with the immune response of the *H-2* recombinant strains shows that in each case the recombinant strain responds to (H,G)-A--L in the same manner as does the donor of the centromeric or *H-2K* region of the recombinant chromosome.

These results once again indicate that *Ir-1* maps in the centromeric (left-

hand) part of the IXth mouse linkage group, either in the *K* region of the *H-2* complex, or somewhere to the left of the *H-2* complex. Because of a shortage of animals, and because the parental $H\text{-}2^d$ and $H\text{-}2^k$ strains differed only in their immune response to (H,G)-A--L, the mouse strains bearing the *H-2* recombinants were tested only with this antigen. If the assumptions given above are correct, one would expect all the *H-2* recombinants in Table III to give a low or variable response to (T,G)-A--L, and a high response to (Phe,G)-A--L. This assumption is currently being tested. The results as given in the table are consistent in that strains bearing the $H\text{-}2^d$ chromosome give a medium and variable response to (H,G)-A--L, while strains bearing the $H\text{-}2^k$ chromosome give a uniformly high response to (H,G)-A--L.

Table IV gives the immune response of strains bearing recombinants between $H\text{-}2^a$ and $H\text{-}2^s$ and $H\text{-}2^a$ and $H\text{-}2^q$. The immune response of strains bearing the parental *H-2* chromosomes is given in the appropriate positions in the table. The results given in this table differ from those given in Tables II and III for two out of the three recombinants listed in Table IV.

The recombinant $H\text{-}2^{tl}$ gives a low response to (T,G)-A--L, a high response to (H,G)-A--L, and a high response to (Phe,G)-A--L. This is similar to the immune response of strains bearing the $H\text{-}2^a$ or the $H\text{-}2^{al}$ recombinant listed in Table III. Since strains bearing $H\text{-}2^s$ are low responders to (T,G)-A--L, (H,G)-A--L, and (Phe,G)-A--L, this indicates that the IXth mouse linkage group bearing $H\text{-}2^{tl}$ responds as does the donor of the right-hand, noncentromeric, (*H-2D*), region of the *H-2* complex. It should be pointed out that the crossover in this case is localized to the left of *Ss* between the *H-2K* locus of $H\text{-}2^s$ and the *H-2D* locus of $H\text{-}2^{al}$. These results are in contrast to those given by mice bearing the $H\text{-}2^{th}$ recombinant derived from a crossover between $H\text{-}2^s$ and $H\text{-}2^a$, with the crossover occurring to the right of *Ss*, between the *Ss* locus and the *H-2D* locus of $H\text{-}2^a$. Mice bearing this *H-2* recombinant respond in a manner similar to mice bearing the $H\text{-}2^s$ allele, once again localizing *Ir-1* to the left of the crossover in either the centromeric part of the *H-2* complex or of the IXth mouse linkage group.

Using the assumptions listed above, the response of the two recombinant $H\text{-}2^t$ alleles can be interpreted in one of two ways. The first is that the crossover giving rise to the $H\text{-}2^{tl}$ recombinant is farther to the left within the *H-2* complex than any of the other crossovers tested, and the fact that mice bearing this chromosome respond as does the donor of the noncentromeric part of the *H-2* complex indicates that *Ir-1* is localized between the *H-2K* locus of *H-2* and the *Ss* locus. Since the $H\text{-}2^{th}$ recombinant was derived from a crossover that occurred to the right of *Ss*, it is to be expected that mice bearing this chromosome would respond as do mice bearing the $H\text{-}2^s$ chromosome. The alternate explanation is that the $H\text{-}2^{tl}$ recombinant is the result of a double crossover event and that the assumptions listed above are incorrect in this respect.

Very strong evidence that the former interpretation is correct is provided by

studies of the immune response of mice bearing the recombinant *H-2^y* chromosome derived from a crossover between *H-2^q* and *H-2^a*. This recombinant, *H-2^y*, is known to be derived from a single crossover event (15) because one of the parental IXth mouse linkage groups involved in the crossover event had

TABLE IV

Immune Response of Strains Bearing H-2 Chromosomes Recombinant Between H-2^a and H-2^s or H-2^a and H-2^q

Strain	*H-2* Type	Crossover diagram*	Immunizing antigen	No. of mice	% Antigen bound		Serum dilution‡	Antigen used for titration§
					Range	Average		
A	a	—	(T,G)-A--L 509	9	5-15	10	1/500	509
A	a	—	(H,G)-A--L 1201	8	61-83	77	1/10	1201
A	a	—	(Phe,G)-A--L 223	10	73-76	75	1/10	223
A.SW	s	—	(T,G)-A--L 509	6	0	0	1/500	509
A.SW	s	—	(H,G)-A--L 905	6	0	0	1/10	905
A.SW	s	—	(Phe,G)-A--L 223	6	<5-27	15	1/10	223
H-2$^{t-Ss^l}$	t^1	K^s | Ss^{s1} D^{s1}	(T,G)-A--L 509	5	<5	<5	1/500	509
H-2$^{t-Ss^l}$	t^1	K^s | Ss^{s1} D^{s1}	(H,G)-A--L 905	13	67-72	70	1/10	905
H-2$^{t-Ss^l}$	t^1	K^s | Ss^{s1} D^{s1}	(Phe,G)-A--L 223	12	8-69	41	1/500	509‖
H-2$^{t-Ss^h}$	t^h	K^s Ss^s | D^s	(H,G)-A--L 905	8	0	0	1/10	905
DBA/1	q	—	(T,G)-A--L 509	10	0	0	1/500	509
DBA/1	q	—	(H,G)-A--L 1201	10	0	0	1/10	1201
DBA/1	q	—	(Phe,G)-A--L 223	10	69-76	74	1/10	223
AQR	y	K^q | Ss^a D^a	(T,G)-A--L 509	6	0	0	1/500	509
AQR	y	K^q | Ss^a D^a	(H,G)-A--L 905	3	55-60	57	1/10	905
AQR	y	K^q | Ss^a D^a	(H,G)-A--L 905	6	36-49	45	1/500	905
AQR	y	K^q | Ss^a D^a	(Phe,G)-A--L 223	3	61-67	64	1/10	223
AQR	y	K^q | Ss^a D^a	(Phe,G)-A--L 223	6	51-63	57	1/500	223

* The notation used here follows the recent relocation of the centromere in the IXth mouse linkage group (references 16–18).

‡ Dilutions for (H,G)-A--L 905 and 1201 and (Phe,G)-A--L 223 were 1/10 to give equimolar amounts of antigen and antiserum with respect to the assay for (T,G)-A--L 509 (1/500 serum dilution).

§ (H,G)-A--L 905 and 1201 gave equal % antigen-bound values in this assay. In addition, both elicited identical immune responses in high-responder and low-responder strains (McDevitt, H. O., and E. Mozes. Unpublished data).

‖ In a few instances, the extensive cross-reactions between antisera to these three antigens were utilized to titer antisera to (H,G)-A--L or (Phe,G)-A--L after the supply of 905-^{3}H, 1201-^{3}H, and 223-^{3}H was exhausted (reference 23).

outside markers to the right (translocation 138, or T138) and left (*T*, brachyury) of the *H-2^q* complex. These outside markers serve to establish that the initial crossover event was, in fact, a single crossover. The immune response of mice bearing *H-2^y* is similar to that of mice bearing *H-2^{tl}* in that it is characteristic of the donor of the *H-2D* locus of the *H-2* complex. Thus, the *H-2^a* mice are

low responders to (T,G)-A--L, high responders to (H,G)-A--L, and high responders to (Phe,G)-A--L. On the other hand, mice bearing *H-2*^{q} (T138,DBA/1) are high responders only to (Phe,G)-A--L. Mice bearing the *H-2*^{v} recombinant respond well to both (H,G)-A--L and (Phe,G)-A--L, as do *H-2*^{a} mice. Since this crossover maps between the *H-2K* locus of *H-2* and the *Ss* locus, *Ir-1* must be a property of the donor of the centromeric (or right-hand) part of the *H-2* complex. Furthermore, since this crossover involved a single exchange, it must be concluded that *Ir-1* maps between *H-2K* and the *Ss* locus.

TABLE V

Results of a Four-Point Cross Designed to Map Ir-1 with Respect to H-2

Recombinants between	Number	Per cent	(Phe,G)- A--L response*	
H-2 - T	57	11.7	See below	
H-2 - tf	19	3.9	Appropriate	14
			Inappropriate (No. 351, No. 489)	2
tf - T	22	4.5	Appropriate	20
			Inappropriate	0
Not localized (infertile in scoring backcross)	16	3.3	Appropriate	11
			Inappropriate	0

Total number of backcross mice scored: 484 (see Fig. 2 for composition of this cross).

* 10 known recombinant mice were not immunized because they died during the long period required for scoring for *tufted* (8–10 wk) and for backcrossing several times to the *tufted* linkage stock or to an inbred strain which is +/+ at the *T* locus. Previous testing established that the *T - tf - H-2*^{q} linkage stock responded to (T,G)-A--L; (H,G)-A--L and (Phe,G)-A--L in a manner identical to the DBA/1 strain.

It should also be pointed out that *H-2*^{tl} and *H-2*th, derived from two separate crossover events, have been extensively analyzed (22) and shown to be identical for all the known serologic specificities possessed by either *H-2*^{a} or *H-2*^{s}. Thus, these two recombinants appear at present to differ only at the *Ss* and *Ir-1* loci. While these serological results must be verified by skin graft testing, they suggest that *Ir-1*, like *Ss* (5), maps near the center of the *H-2* complex, but is not itself a transplantation antigenic specificity.

Localization of the Ir-1 Locus by Genetic Mapping.—

Independent verification of the localization of *Ir-1* near the center of the *H-2* complex between *H-2K* and the *Ss-Slp* complex was obtained by a four-point linkage test. The genotype of the original linkage stock and the A.SW strain, as well as the genotype of the parents used in the test cross, was previously described (Materials and Methods, and Fig. 2). As outlined in the Materials and Methods section, offspring of the cross diagramed in Fig. 2 were scored with respect to *T*, *tf*, and *H-2*. By determining the genotype of known recombinant mice, the cross-

overs could be located either between *T* and *tf*, or between *tf* and *H-2*. In some cases, putative crossovers could not be progeny tested because of infertility. All of the proven recombinant mice were then immunized with (Phe,G)-A--L. Since the cross was on an A.SW background, (nonresponders to (Phe,G)-A--L), recombinant mice bearing $H\text{-}2^q$ would be expected to be high responders to (Phe,G)-A--L, while recombinant mice lacking $H\text{-}2^q$ would be expected to be low responders to (Phe,G)-A--L.

TABLE VI

Immune Response of Recombinant Mice from Mapping Study and Their Progeny

Animal No.	*H-2* genotype	Reaction with AS-348 (anti-H-2.17,30)	Immune response to (Phe,G)-A--L*
351	q/s	+	0
Backcross 1 (351 × A.SW)			
716	s/s	−	0
717	s/s	−	0
718	s/s	−	0
719	q/s	+	0
720	q/s	+	0
721	q/s	+	0
722	q/s	+	0
489	q/s	+	12
Backcross 1 (489 × A.SW)			
1122	s/s	−	N.T.
1123	q/s	+	N.T.
1124	s/s	−	N.T.
Backcross 2 (1123 × A.SW)			
1449	s/s	−	0
1450	q/s	+	0
1451	q/s	+	8
1452	q/s	+	N.T. (mated)
1453	s/s	−	0
1454	q/s	+	0
1455	q/s	+	N.T. (mated)
1456	s/s	−	0
1457	q/s	+	N.T. (mated)

* Mice immunized with (Phe,G)-A--L 223 and titered with (T,G)-A--L 509 at 1/500 serum dilution.

Results of this mapping study are given in Table V. Of 484 offspring, 57 (11.7%) recombinants between *T* and *H-2* were actually detected. This is consistent with the expected frequency of 14%, particularly since the

heterozygous parents included both male and female mice. (The predicted rate of 14% recombination is for females and is known to be lower in males.) Of the 41 recombinants that could be further localized, approximately half occurred between *T* and *tf* and half between *tf* and *H-2*. This again agrees with the expected equal frequencies of crossovers between these three loci predicted by the equal mapping distance (seven map units) between *T* and *tf* and between *tf* and *H-2*. As can be seen in the last column of Table V, two mice, which were derived from a crossover between *tf* and *H-2*, gave an inappropriate immune response to (Phe,G)-A--L. Both of these mice appeared to carry the $H\text{-}2^q$ chromosome and were low responders to (Phe,G)-A--L. Presence of the $H\text{-}2^q$ allele was established by reaction with antiserum No. AS-348, which was expected to have as its principal specificity H-2.17, a specificity of the *H-2K* locus of the $H\text{-}2^q$ complex, but probably also contains antibodies to H-2.30, a specificity of the *H-2D* locus of $H\text{-}2^q$.

TABLE VII

Summary of Serological Data on (No. 351 × C3H.B10)F₁ Mice

Test cell	*H-2* type	Anti-17 (C3H.B10 × AKR.M) α-C3H·Q		Anti-19 (B10 × A) α-A.SW		Anti-30 (B10.A × AKR) α-AKR.M		Anti-11 (D2 × BF) α-CR
		CT‡	HA§	CT	HA	CT	HA	HA
351 × C3H.B10	rec/b	0*	0	80*	2560*	40*	640*	0*
C3H.Q	q/q	160	160	0	0	40	320	80
B10.S	s/s	0	0	160	640	0	0	0

* Confirmed by in vivo absorption.

‡ CT = cytotoxic test. Reciprocal of highest dilution giving a positive result.

§ HA = hemagglutination.

Table VI shows the results of progeny testing of these two animals (Nos. 351 and 489) with respect to the presence in their offspring of the H-2.17 specificity, and their immune response to (Phe,G)-A--L. As can be seen from this table, both the reaction with AS-348 and a low response to (Phe,G)-A--L were inherited in the progeny of these two animals.

These results, of themselves, suggested recombination between the $H\text{-}2K^q$ locus and the *Ir-1* locus. Further characterization of this recombinant required testing recombinant animals for the presence or absence of the *H-2D* specificities of the $H\text{-}2^q$ complex.

This was done by several serological tests and by F_1 skin graft tests. Table VII is a summary of the serological data on offspring of recombinant no. 351 (whose progeny testing was presented in Table VI). The antisera used and their specificities are shown in Table VII. The results are given as titers against test cells from the indicated sources in the cytotoxicity test or the hemagglutination

test. Specificities H-2.17 and H-2.11 are specificities of the *H-2K*^{q} allele. Specificity 30 is a private specificity of the *H-2D*^{q} allele. Specificity 19 is a private specificity of the *H-2K*^{s} allele. Table VII shows the results with the progeny of recombinant No. 351, as well as with animals of the C3H.Q strain, which is congenic with C3H, but carries *H-2*^{q}, and the B10.S strain, which is congenic with C57BL/10, but carries *H-2*^{s}. As can be seen, recombinant No. 351 lacks specificities H-2.17 and H-2.11 of the *H-2K*^{q} allele. These specificities appear to be replaced with specificity H-2.19 from the *H-2K*^{s} allele. The No. 351 progeny do carry specificity H-2.30 from the *H-2D*^{q} allele. Therefore, recombinant

TABLE VIII

F_1 Skin Graft Tests of Recombinant No. 351

Experiment	Donor (*H-2* type)	Recipient (*H-2* type)	Surviving grafts	Mean survival time
				days
1	C3H.Q ($H\text{-}2^q$)	(C3H × DBA/1)F_1 ($H\text{-}2^k/H\text{-}2^q$)	10/10	>100
2	C3H.Q ($H\text{-}2^q$)	(C3H × (351 × A.SW)BC) ($H\text{-}2^k/H\text{-}2^s$ or $H\text{-}2^k/H\text{-}2^{rec.}$*)	0/16	12.7
3	C3H.Q ($H\text{-}2^q$)	(C3H.B10‡ × (351 × A.SW)BC) ($H\text{-}2^b/H\text{-}2^s$ or $H\text{-}2^b/H\text{-}2^{rec.}$)	0/24	11.1
4	C3H.Q ($H\text{-}2^q$)	(C3H.B10‡ × (351 × A.SW)BC) ($H\text{-}2^b/H\text{-}2^s$ or $H\text{-}2^b/H\text{-}2^{rec.}$)	0/26	<14§

* $H\text{-}2^{rec.}$ is used to designate the *H-2* recombinant allele of No. 351.

‡ Several F_1 skin graft experiments (D. C. Shreffler, unpublished data) have shown that C3H.B10 is entirely histocompatible with C3H and C3H.Q, except for the *H-2* differences.

§ All grafts were rejected by 14 days.

No. 351 and its progeny appear to carry a IXth mouse linkage group with a crossover within the *H-2* complex between the *H-2K* and *H-2D* loci. As indicated by the asterisks in Table VII, the specificities assigned by serological testing were confirmed by in vivo absorption of the various anti-*H-2* specificities in the respective antisera.

In addition, progeny of recombinant No. 351 were subjected to an F_1 skin graft test. In this test, progeny of several backcrossed generations, in which offspring of recombinant No. 351 were successively backcrossed to A.SW, were crossed with C3H or C3H.B10, a strain congenic with C3H, but carrying $H\text{-}2^b$. The progeny of this cross then carried $H\text{-}2^b$ or $H\text{-}2^b$ in association with either the *H-2* recombinant or $H\text{-}2^s$ from the A.SW line. All animals were skin grafted with skin from C3H.Q animals, which are congenic with C3H and C3H.B10, except for the *H-2* complex, where they carry $H\text{-}2^q$. If the *H-2* chromosome in No. 351 is indistinguishable from $H\text{-}2^q$, then skin from the C3H.Q line should be accepted by the progeny of the cross [(351 × A.SW) backcross × C3H or C3H.B10].

As shown in Table VIII, in three separate series of tests all the progeny rejected the C3H.Q skin grafts within 11.1–12.7 days. This is compatible with a difference at the *H-2* locus and indicates that the $H\text{-}2^q$ specificities found in recombinant No. 351 are incomplete and do not represent an intact $H\text{-}2^q$ allele.

Thus, the results of both the serological tests and the F_1 skin graft tests are concordant in showing that the *H-2* chromosome carried by the progeny of recombinant No. 351 lack the $H\text{-}2K^q$ specificities, and carry the $H\text{-}2K^s$ specificities. This means that the initial indication of recombination between $H\text{-}2^q$ and *Ir-1* in animal No. 351 was actually due to a crossover within the *H-2* complex. This result is thus completely consonant with the results of immunizing animals bearing known *H-2* recombinants, indicating that the *Ir-1* locus is near the center of the *H-2* complex. (Similar tests are currently being carried out on the progeny of recombinant No. 489 in an effort to establish

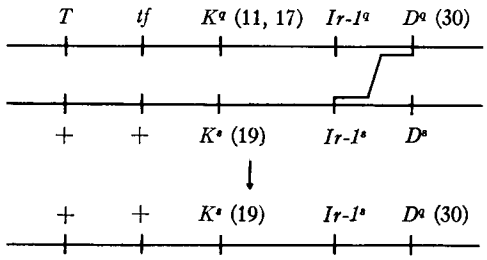

FIG. 3. A schematic diagram of the crossover event giving rise to recombinant No. 351. This diagram is based on the data in Tables VII and VIII.

that this animal also represents a crossover within the *H-2* complex, rather than between *H-2* and *Ir-1*.) This crossover is diagramed in detail in Fig. 3.

DISCUSSION

The results presented in this paper indicate that the *Ir-1* locus, controlling the quantitative immune response to a series of branched, multichain, synthetic polypeptide antigens, maps within the *H-2* complex, somewhere between the *H-2K* locus controlling the *K* set of histocompatibility antigenic specificities, and the *Ss* locus controlling the level of a serum alpha globulin (see Fig. 1). The position of *Ir-1* within the *H-2* complex is indicated in the first place by the results of immunizing strains of mice homozygous for 11 different known recombinant *H-2* types. The crossovers giving rise to these *H-2* recombinants occurred between the *H-2K* locus and the *Ss* locus, or between the *Ss* locus and the *H-2D* locus. The results with nine of the eleven *H-2* recombinants indicated that *Ir-1* was to the left of the crossover. Results with two of the eleven recombinant *H-2* chromosomes indicated that *Ir-1* was to the right of the cross-

over. Both of these *H-2* recombinants were derived from crossover events which took place between the *H-2K* locus and the *Ss-Slp* locus. Since one of these recombinants, $H\text{-}2^u$, was derived from a known single crossover event (proven by outside markers to the left and right of the *H-2* complex), the possibility that both of these recombinants were the result of a double crossover has been almost completely ruled out.

The results of the four-point linkage test are entirely consistent with the above conclusion. In 484 offspring, only two putative recombinants between *H-2* and *Ir-1* could be detected. Although there is a possibility that this crossover incidence is slightly low (due to the 10% failure of penetrance of the *T* allele), it must be noted that a recombination rate of 0.4% between *H-2* and *Ir-1* is of the order of magnitude previously established for crossover frequencies within the *H-2* complex. Of the two putative crossovers between *H-2* and *Ir-1*, extensive testing of mouse No. 351 has established the event as a crossover within the *H-2* complex in which the recombinant chromosome carries the *H-2K* specificities of $H\text{-}2^s$ and the *H-2D* locus of $H\text{-}2^q$. This recombinant *H-2* chromosome is thus similar to nine of the eleven previously tested *H-2* recombinants in indicating that *Ir-1* is associated either with *H-2K* or the centromeric part of the chromosome. The second tentative recombinant between *H-2* and *Ir-1* is currently being tested.

Since it is now clear that there are a great many histocompatibility-linked specific immune response genes (3), the significance of the finding that one of these genes, *Ir-1*, maps within the *H-2* complex must be considered. The nature of the relationship between *H-2* and *Ir-1* must also be considered in the light of possible mechanisms of gene action of the histocompatibility-linked specific immune response genes. This subject has been discussed in detail elsewhere (3) and will not be repeated here, except to point out that the presently available evidence suggests that immune response genes affect antigen recognition, perhaps at the level of thymus-derived, antigen-reactive lymphocytes, or possibly at the level of both thymus-derived and bone marrow–derived (antibody-forming precursor) lymphocytes. Since *Ir-1* maps inside the *H-2* complex, it is logical to consider whether this gene(s) controls some cell surface structure which is responsible for antigen recognition by one or several types of immunocompetent cells.[3]

[3] It should be pointed out that *Ir-1* probably does not represent an immunoglobulin variable region gene because it has been shown previously (23) that *Ir-1* is not linked to the mouse immunoglobulin allotypes (which are localized in the heavy chain constant region linkage group), and because of the findings in rabbits (Prahl, J. W., W. J. Mandy, G. S. David, M. W. Steward, and C. W. Todd. 1970. Participation of allotypic markers in rabbit immunoglobulin classes. *In* Protides of the Biological Fluids (XVIIth Colloquium), Bruges, 1969. Pergamon Press Ltd., Oxford, England. 125–130.) that the heavy chain constant and variable region are genetically linked. This does not rule out the possibility that *Ir-1* represents a light chain variable region gene. The latter possibility seems less likely, since a major part of the specificity of antibodies appears to be contributed by the heavy chain variable region.

In the present state of our knowledge, the possible relationship between *H-2* and *Ir-1* can be broken into two broad categories. The first is that *Ir-1* represents some type of chemically specific interaction between *H-2* antigenic specificities and classical immunoglobulin receptors on the surfaces of immunocompetent cells. The second possibility is that *Ir-1* is completely unrelated to the *H-2* complex but maps in the same part of the IXth mouse linkage group because it controls some other cell surface structure which is intimately involved with specific antigenic recognition. If this is the case, the nature of the *Ir-1* gene product must at the present time remain a matter of speculation. However, further mapping studies designed to localize the other *H-2* linked specific response genes within the IXth mouse linkage group in general, and within the *H-2* complex in particular, can be of considerable value in differentiating between these two broad categories of possible explanations for the mechanism of action of the specific immune response genes.

If the first explanation given above is correct, one would expect that different immune response genes would be a property of different *H-2* antigenic specificities, and that therefore separate immune response genes would map with the *H-2K* locus, or with the *H-2D* locus. On the other hand, if the second possibility is correct, and the specific immune response genes are the result of a separate and distinct antigenic recognition system, possibly unrelated to immunoglobulin, then one might expect them all to map in the same narrow region between *H-2K* and *Ss-Slp*, or possibly between *H-2K* and *H-2D*. Since there are now a large number of antigens under this type of histocompatibility-linked genetic control, mapping studies analogous to the ones presented here could provide a basis for a tentative choice between the two alternatives given above.

A second approach to the resolution of this choice could be provided by the detection of two recombinant *H-2* chromosomes which were histocompatible but differed at the *Ir-1* locus. (A systematic search for such differences among the known inbred strains bearing known *H-2* alleles has been unproductive [see reference 1].) As mentioned above, the $H\text{-}2^{tl}$ and the $H\text{-}2^{th}$ recombinant alleles appear, by serologic testing, to fulfil these criteria. Definitive proof of this possibility will require the production of congenic strains which carry the two recombinant alleles on an identical genetic background, after which reciprocal exchange of skin grafts will prove identity or nonidentity of the recombinant *H-2* alleles.

There is tentative evidence to suggest that the second of the two possibilities presented above is the correct one. Bailey (24) has shown that the genetic control of the ability to reject the *Y* transplantation antigen is associated with the centromeric, or *H-2K*, region of the *H-2* complex. Lieberman (25) has shown that the genetic control of the ability to produce anti-γA allotype antibody is also a property of the *K* region of the *H-2* complex. Finally, Merryman (personal communication) has shown that the ability of mice to respond to the linear

synthetic polypeptide GAT_{10} is also a property of the *K* region of the *H-2* complex. Vaz et al. (26) have also shown that the ability to respond to low doses of ovomucoid and bovine γ-globulin is a property of the *K* region of the *H-2* complex. In each of these cases, localization was accomplished through the use of *H-2* recombinants produced by Stimpfling from crossovers between $H\text{-}2^a$ and $H\text{-}2^b$. These results are identical to those given with (T,G)-A--L and (H,G)-A--L, and suggest that for the five different specific immune response genes so far tested, all appear to be localized to the same part of the IXth mouse linkage group, and therefore, presumably, to the same part of the *H-2* complex. A definitive answer to this question will require completion of the mapping studies for the specific immune response genes already listed above, and extension of these studies to several other specific immune response genes linked to the *H-2* complex. If these results indicate that the immune response genes all map between the *H-2K* locus and the *Ss-Slp* complex, the nature of the gene product controlled by a locus such as *Ir-1* remains a problem for further investigation.

Note Added in Proof.—Serologic analysis of the second recombinant between *H-2* and *Ir-1* (No. 489) in the mapping study has shown that this recombinant *H-2* chromosome has specificity 19 from $H\text{-}2^s$ and specificity 30 from $H\text{-}2^q$. This recombinant chromosome is thus similar to recombinant No. 351, as would have been predicted."

SUMMARY

Eleven strains of mice bearing recombinant *H-2* chromosomes derived from known crossover events between known *H-2* types were immunized with a series of branched, multichain, synthetic polypeptide antigens [(T,G)-A--L, (H,G)-A--L, and (Phe,G)-A--L]. Results with nine of the eleven *H-2* recombinants indicated that the gene(s) controlling immune response to these synthetic polypeptides (*Ir-1*) is on the centromeric or *H-2K* part of the recombinant *H-2* chromosome. Results with two of the eleven recombinant *H-2* chromosomes indicated that *Ir-1* was on the telomeric or *H-2D* part of the recombinant *H-2* chromosome. Both of these recombinants were derived from crossovers between the *H-2K* locus and the *Ss-Slp* locus near the center of the *H-2* region. One of these recombinants, $H\text{-}2^u$, was derived from a known single crossover event. These results indicate that *Ir-1* lies near the center of the *H-2* region between the *H-2K* locus and the *Ss-Slp* locus.

The results of a four-point linkage test were consistent with these results. In 484 offspring of a cross designed to detect recombinants between *H-2* and *Ir-1*, only two putative recombinants were detected. Both of these recombinants were confirmed by progeny testing. Extensive analysis of one of them has shown that the crossover event occurred within the *H-2* region. (Testing of the second recombinant is currently under way.) Thus, in the linkage test, recombinants between *H-2* and *Ir-1* are in fact intra-*H-2* crossovers. These results permit assignment of *Ir-1* to a position between the *H-2K* locus and the *Ss-Slp* locus.

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