Genetic Control of the Antibody Response: Relationship between Immune Response and Histocompatibility (H-2) Type

Abstract. *The immune responses of inbred mice to a related series of three synthetic polypeptide antigens are genetically controlled traits which are closely correlated with the genotype for the major histocompatibility* (H-2) *locus. All strains of the same* H-2 *type exhibit the same pattern of immune response, independent of the remainder of a given strain's genetic background. There is marked antigen-specific polymorphism between strains of different* H-2 *types with respect to their patterns of response.*

Genetic control of the immune response to specific antigens has been demonstrated with several antigens in inbred mice and guinea pigs (*1*). Recent results (*2*) have shown that the ability of mice to respond to a series of branched, multichain, synthetic polypeptide antigens is a genetic trait which can be transferred with "responder" spleen cells, and which is closely associated with the major histocompatibility (*H-2*) locus in the IXth mouse linkage group. The antigens used in these studies (*3*) are built upon a branched carrier of poly-D,L-alanyl--poly-L-lysine (A--L), and bear a restricted range of antigenic determinants on the tips of the poly-D,L-alanyl side chains. These side chain tips are made up of tyrosine and glutamic acid, poly-(tyr,glu)-poly-D,L-ala--poly-L-lys [(T,G)-A--L], histidine and glutamic acid [(H,G)-A--L], or phenylalanine and glutamic acid [(P,G)-A--L]. It has already been established (*2*) that C57 mice (which are *H-2*^{b}) respond well to (T,G)-A--L and poorly to (H,G)-A--L, while CBA mice (which are *H-2*^{k}) respond poorly to (T,G)-A--L and well to (H,G)-A--L. In both cases, the ability to respond well was shown, by the use of a segregating generation (backcross), to be very closely linked to the *H-2* locus. Both of these strains respond well to (P,G)-A--L, so that linkage studies could not be carried out with this antigen in these strains.

The present study explores the relationship between histocompatibility-2 (*H-2*) genotype of 33 mouse strains, which among them carry eight different *H-2* alleles, and the ability to respond immunologically to the three synthetic antigens described above. The mice were immunized as described previously, with that dose of antigen which had been shown to elicit a maximal antibody response in a high-responding strain (*3*). Antibody response was measured by an antigen-binding capacity assay (*2*), by using iodinated or tritiated antigens where appropriate (*3*), and modified for use with very small amounts of antiserum and antigen (*2*).

The response of a large number of inbred mouse strains to (T,G)-A--L is shown in Table 1. There is a striking uniformity of response to (T,G)-A--L, which is correlated with the *H-2* type of the strain and not with the rest of the strain's genetic background. For example, all *H-2*^{b} mice respond well to (T,G)-A--L, including mice of strain C3H.SW, which is essentially isogenic or genetically identical with strain C3H, except for a difference at the *H-2* locus. C3H mice are *H-2*^{k} and respond poorly to this antigen, as do the other *H-2*^{k} strains listed. *H-2*^{b} strains respond well to (T,G)-A--L; *H-2*^{d} strains vary a great deal in response, and all the other *H-2* type strains tested respond poorly to this antigen.

Immunization of selected strains of seven different *H-2* types with these three branched, synthetic polypeptide antigens demonstrated the same uniformity of response to each antigen within a particular *H-2* type, but an apparently independent variation between *H-2* types in their pattern of response to these three antigens. These results are shown in Table 2. The relationship between immune response to (T,G)-A--L and *H-2* type has already been commented upon. When these same strains of mice are immunized with (H,G)-A--L, *H-2*^{a} and *H-2*^{k} animals respond well; *H-2*^{d} animals respond variably; and all the other *H-2* type strains tested respond poorly. This pattern is radically different from that seen with (T,G)-A--L. When these same strains are immunized with (P,G)-A--L, a completely different pattern of response is obtained. Every strain tested with this antigen responded well with the exception of those strains which are *H-2*^{s}, both of which responded very poorly to this antigen. The two *H-2*^{s} strains responded poorly to all three antigens, while DBA/1 mice (*H-2*^{q}) responded well only to (P,G)-A--L, and all the other *H-2* type strains tested responded well to two out of three of the antigens used. So far, no strains have been identified which respond well to all three antigens, or which respond well only to (T,G)-A--L or only to (H,G)-A--L. The failure of *H-2*^{s} strains to respond to all three of these antigens is clearly a property of the *H-2*^{s} allele, since the A.SW strain (*H-2*^{s}) is congenic with the A and A.BY strains, differing from them only at the *H-2* locus.

The identification of a strain which responded poorly to (P,G)-A--L permitted formal tests to establish whether

Table 1. The antibody response of different inbred mouse strains of eight different *H-2* types to (T,G)-A--L. The antibody response is listed as the average percentage of antigen bound. A low percentage of antigen bound value indicates a low antibody response, and a high percentage of antigen bound value indicates a high antibody response. For purposes of rough quantitation, CBA and C57 mice differ approximately tenfold in their total antigen binding capacity to this antigen (*6*) and approximately fivefold in the amount of precipitating antibody which they make to this antigen. CBA mice make 80 to 90 μg of antibody per milliliter, while C57 mice make approximately 400 μg of antibody per milliliter (*6*). For each strain listed, five males and five females approximately 2 to 4 months of age were immunized.

Strain	*H-2* type	Antigen bound (%)	Range
A/J	*a*	10	5-15
A.BY	*b*	78	62-87
C57	*b*	69	53-82
C57BL/6	*b*	42	21-64
C57BL/10	*b*	58	38-86
C57L	*b*	54	21-71
D1.LP	*b*	59	40-95
C3H.SW	*b*	79	52-91
129/J	*b*	52	17-79
LP/J	*b*	69	56-80
CWB	*b*	65	53-82
BALB/c	*d*	28	0-55
C57BL/Ks	*d*	46	16-70
B10.D2	*d*	22	0-39
DBA/2	*d*	34	11-53
WH.RE	*d*	50	0-74
B10.D2 (old)	*d*	17	0-43
B10.D2 (new)	*d*	15	10-24
NZB	*d*	32	10-52
CBA*	*k*	12	0-27
C3H/HeJ	*k*	17	9-26
C57BR/cd	*k*	8	4-13
C58/J	*k*	6	4-9
B10.BR	*k*	6	2-14
DBA/1	*q*	6	4-12
RIII	*r*	8	2-13
SJL	*s*	5	3-7
A.SW	*s*	0	
WB/Re	*w*	0	
SWR	?	6	
B10-T	?	10	0-32
BRT	?	14	0-39
Random-bred Swiss mice	?	0	

* These animals were produced from CBA's maintained for many years at the National Institute for Medical Research, Mill Hill, London.

the ability to respond to this antigen is also under genetic control and linked to the *H-2* locus in a manner similar to that already described for (T,G)-A--L and (H,G)-A--L (*2*). A cross was employed [DBA/1($H\text{-}2^q$) × SJL($H\text{-}2^s$)] which utilized strains entirely different from those used for the original linkage studies and which took advantage of the fact that by the use of this cross we could test linkage in two strains, neither of which responded to (T,G)-A--L *or* (H,G)-A--L. The results of this linkage test are shown in Fig. 1. The results indicate close and perhaps complete linkage between the $H\text{-}2^q$ allele and the ability to respond well to (P,G)-A--L.

These results, taken together, show a remarkable and regular correlation between *H-2* type and antibody response to these synthetic polypeptide antigens. Immune response to each of these three synthetic polypeptide antigens is a genetically controlled trait which is closely linked to the *H-2* locus, and appears to vary independently with respect to ability to respond to the other two synthetic polypeptide antigens. Such a picture is compatible either with multiple loci controlling the ability to respond to these antigens (all closely linked to the *H-2* locus), or with multiple alleles (five or more) at a single locus controlling the immune response to these antigens.

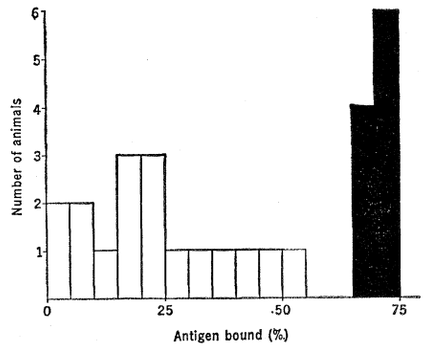

Fig. 1. The immune response of a population of (DBA/1 × SJL) F_1 × SJL mice to (P,G)-A--L. Immunization and antibody titrations were carried out as already described (*3*). *H-2* typing was done by hemagglutination (*7*). Animals which carry the $H\text{-}2^q$ allele donated by the DBA/1 strain are indicated by the black bars. Animals which lack the $H\text{-}2^q$ allele are indicated by white bars.

The ability to respond to (T,G)-A--L can be transferred with "responder" spleen cells (*2*) and thus appears to be due to a gene directly related to the process of antibody formation. However, the exact mechanism of action of the gene or genes controlling the response to these antigens is unknown. The possibility that the animals' *H-2* antigens cross-react with these synthetic antigens (so that the genetic control is a form of "cross-tolerance") is highly unlikely, since it has been shown that the ability to respond well to (T,G)-A--L can be transferred with C57 fetal liver cells (*4*). If cross-tolerance were responsible for this phenomenon, the C57 fetal liver cells should be rendered tolerant, and therefore nonresponsive, in a nonresponsive host.

However, the close and regular association between *H-2* allele and pattern of response raises the possibility that a cell's own complement of surface antigens may predetermine in some way the type of exogenous antigens with which it may interact. An effect of this type could conceivably be exerted at several steps in the process of antibody formation, and possibly on several different cell types. Lilly (*5*) has recently shown that susceptibility to murine leukemia is also closely linked to the *H-2* locus. It is not yet clear whether this susceptibility is mediated through a deficiency in antibody response, or is a membrane effect of the type referred to above. Further understanding of the relationship between *H-2* type and immune response will be possible when this type of genetic control can be localized to a particular cell type (lymphocyte, thymocyte, macrophage), and when the gene(s) can be more accurately mapped within, or adjacent to, the *H-2* locus.

Table 2. The response of inbred strains of mice to three synthetic polypeptide antigens. In each case, the maximal immunizing dose of antigen was given in Freund's adjuvant, followed by an aqueous injection of the antigen 3 weeks later. The results are expressed as average percentage of antigen bound. For those antigens not containing tyrosine, the radioactive labeling reagent was tritiated acetic anhydride of high specific activity.

Strain	*H-2* type	(T,G)-A--L			(H,G)-A--L			(P,G)-A--L		
		Antigen bound (%)	Range	No. of mice	Antigen bound (%)	Range	No. of mice	Antigen bound (%)	Range	No. of mice
A/J	*a*	10	5-15	9	77	61-83	8	75	73-76	10
A.BY	*b*	78	62-87	9	0		6	73	73-75	5
C57	*b*	69	53-82	10	< 5	0-16	22	69	67-71	9
D1.LP	*b*	59	40-95	10	< 5	0-9	10	73	72-73	6
C3H.SW	*b*	79	52-91	10	5	0-13	8	73	70-74	6
BALB/c	*d*	28	0-55	8	42	15-63	10	72	68-75	9
DBA/2	*d*	34	11-53	10	11	0-27	10	65	53-74	6
CBA†	*k*	12	0-27	10	70	39-77	9	71	69-72	7
C3H/HeJ	*k*	17	9-26	10	71	61-82	8	74	72-75	10
B10.BR	*k*	6	2-14	10	68	60-83	10	71	69-74	10
AKR	*k*				70	60-78	10	73	72-74	8
DBA/1*	*q*	6	4-12	8	0		10	74	69-76	10
SJL	*s*	5	3-7	10	5	0-18	10	13	0-39	10
A.SW	*s*	0		6	0		6	15	6-22	5
WB/Re	*w*	0		10	0		10	49	37-62	5
SWR	?	0		10	0		10	71	71	2

* A second $H\text{-}2^q$, tufted (*tf*), short-tail (*T*) linkage strain responded in a manner identical to DBA/1 mice. † These animals were produced from CBA's maintained for many years at the National Institute for Medical Research, Mill Hill, London.

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References and Notes

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