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Immune Response to Calf Collagen Type I in Mice: A Combined Control of *Ir-1A* and non *H-2* Linked Genes

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Abstract. The genetically controlled immune response to calf skin collagen type I in mice could be demonstrated to be governed by at least two genes. One is linked to the *H-2* complex and located within the *IA* subregion. High-responder alleles are $H\text{-}2^b$, $H\text{-}2^f$, and $H\text{-}2^s$. The other gene(s) is not linked to the *H-2* complex and high-responder allele(s) are found in the genome of B10 mice but not in the genome of DBA mice. There are strong indications that the *Ir-1A* gene controls the response at the T-cell level, whereas it is assumed that the background gene(s) control the immune response at a different level.

Introduction

Since collagen offers several immunochemical advantages (reviewed by Crumpton 1974, Furthmayr and Timpl 1975) compared to other native proteins for which genetic control of immune responsiveness has been described, a detailed investigation of possible differences in response potential was made using congenic and recombinant mouse lines. In previous studies (Hahn *et al.* 1975) we presented evidence for the *H-2* linkage of the major gene controlling the immune response to calf skin collagen type I and demonstrated that the *H-2* linked immune response is T-cell dependent (Nowack *et al.* 1975 b). This report will provide data identifying the gene locus governing the response to collagen within the *H-2* complex and the distribution of high- and low-responder alleles among inbred strains of mice. Furthermore, there is some evidence that the immune response to collagen is controlled by addtitional genes, not linked to *H-2*.

Materials and Methods

Animals. All animals were obtained from the Jackson Laboratory, Bar Harbor, Maine, except B10.A(4R) and A.TL mice, which were maintained in our colony. Mice were used for immunization between 6 and 9 weeks of age.

Immunization. Purified acid-extracted collagen from calf skin (collagen type I), procollagen from dermatos-paractic calves, and the immunization schedule were the same as described previously (Hahn *et al.* 1975). Briefly, the first injection was given together with complete Freund's adjuvant subcutaneously and was followed 4 weeks later by an intraperitoneal booster. Blood was collected

7 weeks after the first injection. Antigen doses ranged from 5 to 500 μg and are indicated for each particular experiment in the tables.

Serological Assays. Antibody titers were determined by passive hemagglutionation and are expressed as $-\log_2$ units (Hahn *et al.* 1975). Results shown in a given table were obtained by using a single batch of coated red cells. Mean titers of groups consisting of responders and non-responders (titer below 2) were calculated assuming a titer of 1 for negatively reacting antisera. Student's *t* test was used for statistical evaluations. Radioimmune assays with iodine-labeled collagen and goat antimouse IgG have been described elsewhere (Nowack *et al.* 1975a).

Results

Previous studies with congenic resistant strains of mice (Hahn *et al.* 1975) showed that classification into high and low responders to collagen type I is possible over a broad range of antigen doses (5–500 μg). The response of males or females was equally low or high, respectively. Crosses between high- and low-responder mice B10 and B10.BR resulted in high-responder offspring only. Backcrosses of the F^1 hybrids to the low-responder B10.BR revealed a nearly equal proportion (24:28) of high- and low-responder offspring, indicating that a single, autosomal dominant gene controls the responsiveness to collagen.

Table 1. Antibody Response to Collagen in Recombinant Mouse Strains[a]

Strain	*H-2* Haplotype	Origin of *H-2* Complex Regions						Antibody Titer	No. of Animals
		K	*IA*	*IB*	*IC*	*S*	*D*	($-\log_2 \pm$S.D.)	
C75BL/10	*b*	*b*	*b*	*b*	*b*	*b*	*b*	6.7±2.7	5
B10.A	*a*	*k*	*k*	*k*	*d*	*d*	*d*	1.8±0.5	4
B10.A(2R)	*h2*	*k*	*k*	*k*	*d*	*d*	*b*	2.6±1.0	8
B10.A(5R)	*i5*	*b*	*b*	*b*	*d*	*d*	*d*	5.5±2.7	10
B10.A(4R)	*h4*	*k*	*k*	*b*	*b*	*b*	*b*	3.1±2.0	5
A.TL	*t1*	*s*	*k*	*k*	*k*	*k*	*d*	2.0±2.2	5

[a] Antigen dose = 5 μg; all female. High-responder alleles are *b* and *s*, low-responder alleles are *a*, *k*, and *d* (see Table 2).

Studies with various congenic recombinant lines, as shown in Table 1, allow one to map the gene controlling the response to collagen in the *IA* subregion of the *H-2* complex. A high response is only observed in recombinants possessing the *IA* subregion of high-responder origin, as in B10.A(5R). Mice possessing high-responder chromosomal segments to the right (B10.A(4R)) or to the left (A.TL) of *Ir-1A* are distinct low responders ($p < 0.025$).

The strain distribution pattern (Table 2) of the immune response control to collagen was studied by injecting a higher dose of antigen (50 μg) to reveal possible differences between strains carrying the same *H-2* haplotype. The high-responder allele is present only in three *H-2* haplotypes, e.g., $H\text{-}2^b$, $H\text{-}2^f$, and $H\text{-}2^s$, except that B10.M mice exhibited a moderate response.

All other strains carry a low-responder allele, although there are significant differences between some low-responder strains. Strains with the $H\text{-}2^q$ haplotype (DBA/1, SWR) were nonresponders even after 500 μg of collagen were injected.

Table 2. Antibody Response to Collagen in Inbred Strains of Mice

Strain[a]	H-2 Haplotype	Antibody Titer vs. Collagen ($-\log_2 \pm$ S.D.)[b]		Responder State[c]
		5 μg	50 μg	
B10.A	*a*	1.3±0.5 (4)	6.4±2.8 (5)	l
A	*a*	N.T.	5.8±4.5 (5)	l
B10	*b*	6.7±2.7 (5)	11.1±1.5 (5)	h
C3H.SW	*b*	N.T.	11.5±2.6 (5)	h
A.BY	*b*	N.T.	12.4±1.3 (5)	h
LP	*b*	N.T.	10.2±1.1 (5)	h
D1.LP	*b*	N.T.	9.8±3.1 (5)	h
B10.D2	*d*	2.3±0.5 (5)	6.8±1.9 (10)	l/m
DBA/2	*d*	2.4±1.7 (6)	1.9±2.0 (5)	l
NZB	*d*	2.1±0.5 (9)	5.1±2.6 (5)	l
B10.M	*f*	7.6±1.0 (5)	8.0±0.7 (5)	h/m
A.CA	*f*	N.T.	9.6±2.0 (5)	h
C3H.JK	*j*	N.T.	1.4±0.6 (5)	l
B10.BR	*k*	3.0±1.6 (10)	4.6±2.3 (11)	l
C3H	*k*	1.7±0.7 (5)	7.7±2.9 (5)	l/m
AKR	*k*	N.T.	6.9±2.4 (5)	l
AKR.M	*m*	N.T.	5.6±4.7 (4)	l
B10.AKM	*m*	1.4±0.6 (5)	6.8±0.5 (5)	l
C3H.NB	*p*	N.T.	1.6±0.6 (5)	l
DBA/1	*q*	<2.0 (5)	<2.0 (5)	n
SWR	*q*	N.T.	<2.0 (7)	n
B10.RIII(71 NS)	*r*	N.T.	7.9±4.6 (4)	m
LP.RIII	*r*	N.T.	4.4±2.5 (5)	l
A.SW	*s*	N.T.	11.4±2.0 (5)	h
SJL	*s*	5.9±4.9 (5)	8.9±1.0 (5)	h
B10.PL	*u*	N.T.	5.8±1.0 (5)	l

[a] All female, except for NZB strain.
[b] No. of animals tested are in brackets; N.T. = not tested.
[c] Responder state is classified as h = high response, m = moderate response, l = low response and n = no response.

DBA/1 mice also did not bind significant amounts of antigen in the radioimmune assay (data not shown). In addition DBA/2 mice exhibited a significant lower response than did B10.D2 mice; this became particularly obvious when 50 μg of collagen were injected. Such differences were also observed for other *H-2* haplotypes, especially when they were compared as to their being on B10 background or not (B10.AKM vs AKR.M, B10.RIII vs LP.RIII, or B10.BR vs C3H – the latter when 5 μg was the dose of antigen). These findings suggested that more than one gene might be involved in mounting a high antibody response to collagen and that this gene(s) is not closely linked to *H-2*. Therefore, a low-responder pair was tested for its ability to overcome the *H-2* linked unresponsiveness when the antigen was injected together with a "carrier", i.e., procollagen.

B10, B10.D2, and DBA/2 mice were injected with procollagen and the sera tested for antibody activity against procollagen and collagen. The results are given in Table 3. All three strains produce high titers against the carrier procollagen; however, DBA/2 mice do not produce antibodies against collagen, although *H-2* identical B10.D2 mice do.

Table 3. Mean Hemagglutination Titers ($-\log_2$) in Sera of B10, B10.D2, and DBA/2 Mice Against Collagen and Procollagen after Immunization with Collagen or Procollagen[a]

Immunogen	Antibody Titer vs.	Strain[b]		
		B10	B10.D2	DBA/2
Collagen	Collagen	6.7±2.7 (5)	2.3±0.5(5)	2.4±1.7 (6)
Procollagen	Collagen	6.0±2.6(13)	6.0±2.8 (15)	2.9±2.9 (15)
	Procollagen	11.8±1.2(13)	11.5±1.9 (15)	11.1±1.8 (15)

[a] Antigen dose = 5 μg.
[b] Number of animals in brackets.
[c] Four animals exhibited a titer of between 6 and 9; the remaining 11 animals had no detectable antibodies (log titer <2).

Since the B10 background genome provided an additional allele for high responsiveness to collagen, we tested a few congenic lines differing for minor H-loci (Table 4). The results show that the high responsiveness of B10 mice is not essentially changed by introducing certain non-H-2 alleles. However, since only $H\text{-}8^b$ is derived from the DBA/2 (nonresponder) genome, it should not be expected to detect any possible linkage to other genetic loci, except *H-8* where there is apparently no obvious effect.

Table 4. Antibody Response to Collagen in Mice Differing for *H*-loci other than *H-2*[a]

Strain	Difference from B10	Origin of Differential *H* Allele[b]	Antibody Titer ($-\log_2$)±S.D.[c]
B10(H-1[c], H-3[a], H-7[a], H-8[a])	–	–	11.1±1.5 (5)
B10.C(41N)	$H\text{-}1^b$	BALB/c	13.0±1.4 (5)
B10.129(5M)	$H\text{-}1^b$	129	10.3±2.8 (5)
B10.LP-*a*	$H\text{-}3^b$	LP	11.8±1.7 (5)
B10.C(47N)	$H\text{-}7^b$	BALB/c	13.0±1.4 (5)
B10.D2(57N)	$H\text{-}8^b$	DBA/2	9.7±0.5 (5)

[a] Antigen dose 50 μg. All female.
[b] According to Klein (1973).
[c] Number of animals given in brackets.

Discussion

A high response to calf collagen type I in mice is apparently governed by at least two genes. One of the governing genes is located within the *IA* subregion of the *H-2* complex, as indicated by the high-responder allele distribution among congenic recombinant strains. This gene appears to be involved in the immune response control at the T-cell level (Nowack *et al.* 1975b) since a carrier-linked

collagen, i.e., its percursor form procollagen, can turn low-responder mice B10.D2 and B10.BR into high responders (Hahn *et al.* 1975). The *Ir-1A* gene controlling the immune response to collagen is not identical to or an allele of other known *Ir-1* located immune response genes (Klein 1975), as the distribution of the high-responder allele among congenic strains is not identical to or mutually exclusive to other *Ir-1* controlled antigens.

The finding of intermediate responders and of significant differences between low-responder strains carrying the same *H-2* haplotype (e.g., between B10.D2 and DBA/2 or between B10.RIII and LP.RIII) suggests additional control by genes not closely linked to the *H-2* complex. This may be due to the fact that collagen is a multideterminant antigen and that the antigenic sites are not repetitive (Nowack *et al.* 1975a). Analysis of the immune response to collagen by injecting procollagen indicates that low responsiveness to collagen in DBA/2 mice cannot be corrected as found for the B10.D2 strain. Since both strains are not different in their response to the globular unit (carrier) peculiar to procollagen, low responsiveness to the collagen (hapten) moiety in the DBA/2 strain, if compared with B10.D2, might not be related to a T-cell function.

Even though it is premature to assign this additional genetic control to B cells, it is reasonable to assume that the B10 background genome carries an additional allele necessary for high responsiveness to collagen. Not only is it possible to investigate B-cell involvement by using the procollagen antigen, but it is also feasible to study other parameters, for example antigen processing. DBA/1 mice, which are non-responders to calf collagen type I from skin, are high responders to bovine collagen type II from cartilaginous tissue (Nowack *et al.* 1975a). Such polymorphic variants of collagen, which presumably do not differ in their physical and metabolic properties (Miller 1973) may, therefore, be excellent models to characterize the complex genetic control of the immune response to proteins.

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