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H-2 genetic control of the response of T lymphocytes to insulins. Priming of nonresponder mice by forbidden variants of specific antigenic determinants

H-2 gene control of the response of T lymphocytes to antigenic determinants of ungulate insulins has been studied. We injected H-2-congenic mice with different insulins emulsified in complete Freund's adjuvant and measured the proliferative response *in vitro* of the draining lymph node cells to various related insulins. Two groups of determinants were antigenic: the A chain loop determinant present on bovine and sheep insulins, but absent on pig insulin, and a pig-associated determinant(s) also shared by bovine and sheep insulins. The responses to these two sets of determinants were controlled by H-2 genes as follows: (a) The pig-associated determinant(s) was antigenic for H-2^d but not for H-2^b or H-2^k mice. (b) The A chain loop determinant was immunodominant over the pig-associated determinant(s) for H-2^d mice. In the presence of the A chain loop determinant, H-2^d mice did not respond to the pig-associated determinant(s) on the same molecule. (c) H-2^d mice did not distinguish between the bovine and sheep variants of the A chain loop that differed by substitution of one amino acid. (d) In contrast, H-2^k mice responded only to the sheep variant, while H-2^b mice responded primarily to the bovine variant of the A chain loop determinant. (e) However, injection of H-2^b mice with "forbidden" sheep insulin primed them for an *in vitro* response to the "permitted" bovine variant of the A chain loop determinant. Similarly, some H-2^k mice injected with forbidden bovine insulin responded *in vitro* to the permitted sheep variant of the A chain loop determinant. Thus, H-2 gene products decided whether the immune response would express an affinity for the specific immunizing antigen itself and/or for a closely related variant.

1 Introduction

The question, how immune response (Ir) genes of the major histocompatibility complex (MHC) work to control the immune response to particular antigenic determinants is as yet unanswered [1]. It has been proposed that Ir-gene products might themselves function as the antigen receptors of T lymphocytes [2], or perhaps influence the generation of diversity of such receptors during the differentiation of T lymphocytes [3]. Another possibility is that Ir genes regulate the processing and/or presentation of antigenic determinants by macrophages to T lymphocytes [4, 5]. According to this notion, Ir genes are not concerned directly with antigen receptors on T lymphocytes; rather, they control immune responses by restricting the availability to the receptors of particular antigenic determinants through the agency of antigen-presenting cells.

Some insight into the mechanism of Ir gene control can be achieved by investigating the immune response of T lymphocytes to various insulins. Knowledge of the structure and amino acid sequences of insulins [6, 7] and the availability of various natural insulins have made it possible to define in molecular terms antigenic determinants on the insulin molecule [4, 8]. Furthermore, responses to such determinants have been shown to be controlled by MHC Ir genes [9–11].

Table 1 shows the amino acid substitutions of pig (PiI), bovine (BoI) and sheep (ShI) insulins compared to mouse insulin. The three ungulate insulins all differ by common substitutions at A 4, B 3 and B 30. Therefore, any antigenic determinants

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Abbreviations: Ir: Immune response **MHC:** Major histocompatibility complex **PPD:** Purified protein derivative of tuberculin **ACL:** A chain loop **PiI:** Pig insulin **BoI:** Bovine insulin **ShI:** Sheep insulin

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Table 1. Amino acid substitutions between mouse insulin, PiI, BoI and ShI

Origin of insulin	Position:	Substituted residues[a] ACL 4	8	9	10	B chain 3	30
Mouse		Asp	Thr	Ser	Ile	Lys	Ser
Pig		Glu	Thr	Ser	Ile	Asn	Ala
Cattle		Glu	Ala	Ser	Val	Asn	Ala
Sheep		Glu	Ala	Gly	Val	Asn	Ala

a) The sequence differences relate to those present on both forms of mouse insulin [7].

for mice associated with these residues would be expected to be shared by the three foreign ungulate insulins. In addition, BoI and ShI differ from mouse and PiI in the A chain loop (ACL). Hence, an immune response to ShI and/or BoI insulins that did not cross-react with PiI could be localized to the ACL.

Keck has studied the response of helper T lymphocytes to carrier determinants on ungulate insulins [9–11]. He found that mice of H-2^b strains responded to BoI, but only poorly to ShI or PiI. H-2^k mice responded only to ShI, while H-2^d mice responded to all three ungulate insulins. Keck also found that some F_1 hybrids between H-2^b and H-2^k mice showed genetic complementation for a response to PiI [11]. Keck interpreted these findings as showing that H-2-linked Ir genes controlled the ability of T lymphocytes to recognize specific determinants of insulins [11]. Rosenthal and his colleagues studied the proliferative response of lymph node lymphocytes from guinea pigs that had been immunized against PiI [4, 8]. In contrast to Keck, they concluded that the simplest explanation for their results was compatible with the concept that the function of Ir genes was expressed in antigen presentation by macrophages.

In this report, we present the results of experiments in which we studied the *in vitro* proliferative response of lymph node lymphocytes from H-2-congenic mice that had been primed *in vivo* by injection of ungulate insulins emulsified in adjuvant. We found that recognition of particular antigenic determinants was influenced by the presence of another determinant on the same insulin molecule. In addition, injection of mice with insulin to which they were hereditary nonresponders led to priming of a secondary *in vitro* response against a variant of the determinant to which they could respond. Thus, H-2 genetic nonresponder mice could recognize "forbidden" determinants. This recognition induced a response directed against a "permitted" variant of the forbidden determinant, but not against the forbidden determinant itself. Thus, H-2 gene products appeared to control the preference of the immune response towards variants of specific antigenic determinants.

2 Materials and methods

2.1 Mice

The Weizmann Institute Animal Breeding Center supplied the H-2-congenic inbred mouse strains C3H.DiSn (H-2^k), C3H.Sw (H-2^b), BALB/c (H-2^d), BALB.B10 (H-2^b) and BALB.C3H (H-2^k). Male or female mice were used at the age of 2–3 months; they were matched for age and sex in each experiment.

2.2 Antigens

Crystalline BoI and ShI were purchased from Schwarz/Mann (Orangeburg, NY). Solutions were prepared by adding 10 mg of crystallized insulin to about 10 ml of phosphate-buffered saline (PBS). Several drops of 0.01 N NaOH were added to dissolve the insulin without raising the pH above 8. The insulins at a concentration of 1 mg/ml were then filtered through a Millipore filter 0.45 μm (Millipore, Bedford, MA). PiI in solution was obtained from Nordisk Insulin-Laboratorium, Copenhagen (as Insulin Leo, Neutral). Purified protein derivative of tuberculin (PPD) was obtained from the Ministry of Health, Israel.

2.3 Immunization

Insulins were emulsified in complete Freund's adjuvant (Bacto-Adjuvant Complete H37 Ra, Difco, Detroit, MI), and mice were injected in each hind footpad with 50 μl of emulsion containing 10 μg of BoI or ShI or 15 μg of PiI. Fourteen days later, the draining popliteal lymph nodes were removed and the proliferative response measured *in vitro*. This dose and time schedule was found to be satisfactory in preliminary experiments of dose response and kinetics.

2.4 Proliferative response

To test the degree of sensitization to insulins, suspensions of cells from popliteal lymph nodes were incubated in RPMI 1640 medium containing 0.5% fresh, normal syngeneic mouse serum, 2-mercaptoethanol (5×10^{-5} M) and gentamycin (40 μg/ml) in flat-bottomed microtiter plates (Costar, Cambridge, MA). To each well, 5×10^5 cells were added in 0.2 ml medium containing 50 μg/ml of PPD or insulin, respectively. After 4 days, tritiated thymidine (2 μCi = 74 kBq, spec. act. 10 Ci/mmol, Nuclear Research Center, Negev, Israel) was added to each well. The cells were harvested onto filter paper 4 h later, using an automatic harvester, and the incorporation of thymidine was measured as the Δcpm (cpm in test cultures minus cpm in control cultures without insulin). Each group represents the pooled lymph node cells of 6–10 mice measured as the mean of three replicate cultures. Standard deviations were usually less than 10% of the mean cpm.

Control groups of mice were injected with PBS emulsified in adjuvant (1 : 1), and their lymph node cells were used in the proliferative response in the presence of the test concentrations of insulins. The Δcpm of these control cultures was subtracted from the Δcpm of test mice that had been injected with insulin in adjuvant. This control was necessary because insulin was observed to stimulate the incorporation of thymidine into lymphocytes obtained from mice that had been injected with adjuvant alone. This control allowed us to separate the effect of insulin as an immunogen from its effect as a nonspecific mitogen. The mitogenic effect of insulin on adjuvant-stimulated lymph node cells never exceeded 20% (it was usually less than 10%) of the specific response to insulin as an immunogen.

Table 2. Responses of mice congenic at H-2^d, H-2^b or H-2^k alleles to BoI, ShI or PiI[a)]

Mouse strain	Genome Non-H-2	H-2	Immunized with	*In vitro* proliferative response Background (cpm)	PPD (Δcpm)	Immunizing insulin (Δcpm)
BALB/c	BALB	d	BoI	3752	37686	68682
			ShI	19341	168906	111112
			PiI	3500	54934	22507
BALB.B10	BALB	b	BoI	6924	227914	94406
			ShI	16860	168902	<0
			PiI	24678	202647	3508
BALB.C3H	BALB	k	BoI	23034	203343	3438
			ShI	19249	235227	42602
			PiI	17944	243785	<0
C3H.Sw	C3H	b	BoI	8333	250084	146345
			ShI	9785	306877	<0
			PiI	21958	281215	<0
C3H.DiSn	C3H	k	BoI	20340	136553	<0
			ShI	9403	111731	19920
			PiI	19054	174769	<0

a) Mice were injected in the hind footpads with 10 or 15 μg of various insulins emulsified in complete Freund's adjuvant. Two weeks later, suspensions of lymphocytes (5×10^5) from the draining popliteal lymph nodes were incubated with 50 μg/ml of PPD or the insulin used for immunization in 0.2 ml of medium. The cells were pulsed with [^{3}H] thymidine on day 4 of culture, and the proliferative response of the lymphocytes was measured as the Δcpm.

3 Results

3.1 H-2 genes control proliferative responses to insulins

Table 2 shows the results of experiments using H-2^d, H-2^b and H-2^k-congenic mice on the BALB background, and H-2^b and H-2^k-congenic mice on the C3H background. Groups of these mice were immunized in the hind footpads with BoI, ShI or PiI in adjuvant, and the proliferative responses of lymphocytes from the draining popliteal lymph nodes were measured 14 days later. All the groups responded to the PPD antigen present in the adjuvant. The BALB/c (H-2^d) mice also responded to the three ungulate insulins. However, the H-2^b mice (BALB.B10 and C3H.Sw) showed a significant response only to BoI. In contrast, the H-2^k mice (BALB.C3H and C3H.DiSn) responded only to ShI. This response of H-2^k mice to ShI was found consistently to be relatively lower than the response of H-2^b mice to BoI in 9 experiments.

The *in vitro* proliferative response has been found to depend on the function of T lymphocytes [12]. The evidence for this in the case of insulin, along with the mapping of the Ir genes within the H-2 complex, will form the substance of another report. In general, these results extend to the proliferative response the conclusions first drawn by Keck regarding H-2 genetic control of helper T lymphocytes [9].

3.2 Immunodominance of the ACL determinant for H-2^d mice

To identify the insulin determinants recognized by responding lymphocytes, we immunizd mice against one ungulate insulin and measured the *in vitro* proliferative response against the various ungulate insulins.

As shown in Table 2, BALB/c (H-2^d) mice responded to each of the three ungulate insulins. This could result from recognition of the common determinant(s) shared by PiI, ShI and BoI (Table 1). In addition, the BALB/c mice could have responded to the foreign ACL determinant present on ShI and BoI, and absent on PiI. To investigate these possibilities, we studied the cross-reactivities of the proliferative response to the ungulate insulins. The results are listed in Table 3.

BALB/c mice, immunized with PiI, responded *in vitro* to PiI, BoI and ShI equally well. This supports the conclusion that the mice could recognize the foreign determinant(s) outside the ACL shared by PiI, BoI and ShI (Table 1). However, immunization of BALB/c mice with either BoI or ShI led to a response to these insulins, but not to PiI (Table 3). Since these insulins differ from PiI only at the ACL (Table 1), it is very likely that the response was to the ACL and not to the other determinant(s) shared with PiI. These findings indicate that the ACL determinants are immunodominant over the other foreign determinants present on the ShI or BoI molecule. The BALB/c mice responded to those other determinants only when immunized against PiI that lacked amino acid differences in the ACL region. Hence, recognition of the determinants shared by the three ungulate insulins was controlled by the presence or absence of the foreign ACL determinant.

As shown in Table 3, we have found that the response of H-2^d mice to PiI was consistently lower than their response to ShI or BoI in 6 experiments. Note also that immunization with either BoI or ShI promoted a proliferative response expressed against either insulin. Therefore, the BALB/c (H-2^d) mice did not appear to distinguish between the BoI and ShI variants of the ACL determinant that differed only by a Gly-Ser substitution at position A9.

3.3 ShI primes nonresponder H-2^b mice for a response to the BoI ACL determinant

Experiments were performed to investigate the specificity with which H-2^b and H-2^k mice recognized the BoI or ShI variants of the ACL determinant. Table 4 shows the results of immunizing H-2-congenic mice with ShI in adjuvant and testing their proliferative response *in vitro* to ShI, BoI or PiI. It can be seen that there was no response to PiI among any of the H-2^b or H-2^k mice. This indicates that these mice did not respond to the PiI determinant(s) also present on BoI or ShI. The H-2^k mice (BALB.C3H or C3H.DiSn) responded only to ShI, but not to BoI *in vitro*. Hence, H-2^k mice recognized the sheep ACL determinant and distinguished between it and the bovine variant from which it differs by substitution of one amino acid (Table 1).

Note the response of the H-2^b mice (BALB.B10 and C3H.Sw). These mice did not respond to the ShI with which they had been immunized. However, they did respond *in vitro* to BoI. The response to BoI primed by injection of ShI to these "nonresponder" strains was markedly weaker than the response to BoI that followed immunization with BoI itself (Tables 2 and 5). Nevertheless, the response represented an almost 3-fold increase in cpm above the background cpm, and

Table 3. The BoI and ShI ACL determinants are immunodominant over the determinants present on PiI for BALB/c (H-2^d) mice[a)]

Immunization of BALB/c mice with	Background (cpm)	*In vitro* proliferative response PPD (Δcpm)	Insulins (Δcpm)	
PiI	3500	54934	BoI	21091
			ShI	19736
			PiI	22507
BoI	3752	37686	BoI	68682
			ShI	56813
			PiI	532
ShI	19541	158906	BoI	111112
			ShI	143744
			PiI	<0

a) Mice were immunized as described in the legend to Table 2 with the 3 insulins, and the proliferative response of the lymphocytes was measured against PPD and each of the insulins.

Table 4. Response of H-2^k or H-2^b mice to immunization with ShI[a)]

Immunization with ShI Strain	Genome Non-H-2	H-2	*In vitro* proliferative response Background (cpm)	PPD (Δcpm)	Insulins (Δcpm)	
BALB.C3H	BALB	k	19279	235227	ShI	52007
					BoI	<0
					PiI	<0
BALB.B10	BALB	b	16860	168902	ShI	<0
					BoI	29005
					PiI	<0
C3H.DiSn	C3H	k	9403	110731	ShI	18220
					BoI	3053
					PiI	<0
C3H.Sw	C3H	b	9785	306877	ShI	<0
					BoI	16035
					PiI	<0

a) Mice were immunized with ShI as described in the legend to Table 2, and the proliferative response of the lymphocytes was measured against ShI, BoI or PiI.

is meaningful in view of the lack of detectable response to either ShI or PiI.

3.4 BoI primes nonresponder H-2^k mice for a response to the ShI ACL determinant(s)

Table 5 shows the results of injecting BoI to the same H-2^b and H-2^k strains illustrated above. The H-2^b mice responded very well *in vitro* to BoI, and also responded somewhat to ShI after priming with BoI. Note that the proliferative response of H-2^b mice to ShI was greater after injection of BoI (Table 5) than after injection of ShI itself (Table 4).

Table 5. Response to BoI ACL determinant of congenic H-2^b or H-2^k mice[a)]

Immunization with BoI Strain	Genome Non-H-2	H-2	*In vitro* proliferative response Background (cpm)	PPD (Δcpm)	Insulins (Δcpm)	
BALB.B10	BALB	b	6924	227914	BoI	94406
					ShI	8525
					PiI	<0
BALB.C3H	BALB	k	19279	235227	BoI	<0
					ShI	<0
					PiI	<0
C3H.Sw	C3H	b	16964	328832	BoI	227146
					ShI	63174
					PiI	3284
C3H.DiSn	C3H	k	20340	156893	BoI	<0
					ShI	20066
					PiI	<0

a) Mice were immunized with BoI as described in the legend to Table 2, and the proliferative response of their lymphocytes was measured against PPD and BoI, ShI and PiI.

H-2^k mice that did not respond to BoI showed a variable cross-reactive response. The BALB.C3H mice responded very well to PPD, but gave no response *in vitro* to any of the three insulins. In contrast, the C3H.DiSn mice did respond to ShI after immunization with BoI. This response was weak but represented a 2-fold increase in cpm above background cpm. The C3H.DiSn mice did not respond to BoI itself, to which they were nonresponders. Hence, similar to the results illustrated above for H-2^b mice (Table 4), some H-2^k genetic nonresponder mice could be primed by the forbidden antigen for a response to a permitted variant of the specific antigenic determinant.

Cross-priming between ShI and BoI was observed in a total of 10 experiments.

4 Discussion

The object of the experiments described in this report was to learn how Ir genes linked to the MHC influenced the response of T lymphocytes to antigenic determinants on ungulate insulins. Our approach was to immunize mice congenic at the H-2 complex with ungulate insulins emulsified in complete Freund's adjuvant and to observe the effect of this *in vivo* priming on the *in vitro* proliferative response of lymphocytes from the draining lymph nodes. Analysis of the antigenic determinants was made by comparing the response to insulins differing at defined residues of the molecule.

We found a complicated relationship between H-2 genotype and selection of antigenic determinants. The response of homozygous mice to PiI was associated with the H-2^d genotype but not with the H-2^b or the H-2^k genotypes. The determinant(s) on PiI could not be identified with certainty but was probably related to those portions of the insulin molecule with amino acid substitutions shared by PiI and BoI or ShI, A4, B3

and/or B 30 (Table 1). This was confirmed by the finding that the response of BALB/c mice to PiI was accompanied by cross-reactivity *in vitro* with BoI and ShI (Table 3).

However, the ability of BALB/c mice to respond to the PiI-associated determinant(s) was inhibited by the presence of an ACL determinant. ShI or BoI which shared all of the PiI-associated substitutions primed a response in H-2^d mice that did not cross-react with PiI. The response was directed only against the ACL portion of the molecule in which both insulins differed from PiI (Table 3). Hence, the ACL can be described as dominant over the PiI-associated determinant(s). Note also that the ShI and BoI variants of the determinant appeared to cross-react to a high degree. In summary, H-2^d mice recognized the PiI-associated determinant(s) only in the absence of the ACL determinant which was immunodominant in both its ShI (Gly) and BoI (Ser) variants.

In contrast to the failure of H-2^d mice to distinguish between the ShI and BoI variants of the ACL determinant, H-2^k and H-2^b mice were exquisitely sensitive to the Gly-Ser substitution at A 9. H-2^k mice ostensibly responded to the A 9 Gly variant, but not to the A 9 Ser variant of the determinant (Table 2). The A 9 Ser variant alone was apparently immunogenic in congenic H-2^b mice. Mice of neither H-2 genotype responded to PiI as an *in vivo* immunogen (Table 2) or after priming with ShI or BoI (Tables 4 and 5). Therefore, the H-2^k and H-2^b mice seemed only to respond to a particular variant of the ACL determinant.

However, the variant to which the mice did not respond was still "recognized", since the forbidden determinant was often able to prime for an *in vitro* response to the permitted variant of the determinant. For example, H-2^b mice injected with ShI did not respond to ShI itself, but did respond to the permitted BoI (Table 4). Furthermore, H-2^k mice were often, but not always, primed to respond to the permitted sheep variant of the ACL determinant by injection of the forbidden bovine determinant (Table 5). Further study is needed to ascertain whether genes outside the MHC modulate the responses of different H-2^k mice.

Cross-recognition of the variant determinants was also observed when H-2^b mice were injected with BoI. In addition to priming the expected response to BoI itself, injection of BoI also primed for a response to ShI which was considerably greater than that activated by injecting H-2^b mice with the forbidden ShI (compare Tables 4 and 5).

Since the congenic strains of mice used differed only at the MHC region, H-2 gene products operationally defined which insulin determinants were forbidden and which were permitted for a proliferative lymphocyte response. In short, we observed that Ir gene products performed two functions: they selected antigenic determinants in a hierarchy of immunodominance, and they decided whether the response would express an affinity for the immunizing antigen or for a related variant. Thus, Ir gene products did not impose absolute rules governing which antigenic determinants of insulin were and which were not recognized by T lymphocytes. Rather, Ir genes seemed to regulate how the immune system attended to determinants that it could recognize potentially.

The results of previous studies [13] strengthen this conclusion. We found that some H-2^k mice could be induced to respond to forbidden BoI by injecting them with syngeneic exudate cells that had been fed with BoI. In contrast, some H-2^b mice were unable to respond to BoI-fed syngeneic cells, although BoI emulsified in adjuvant was highly immunogenic for H-2^b mice (Tables 2 and 5). Hence, the phenotypic expression of Ir genes for the response to insulins was found to be variable and influenced by the mode of immunization as well as by the array of antigenic determinants on the molecule.

Another example of cross-priming by forbidden determinants was observed by Sercarz et al., who studied the H-2 genetic control of the immune response to fowl lysozymes [14]. They found that H-2^b mice injected with chicken lysozyme responded very poorly to chicken lysozyme itself. Nevertheless, injection with chicken lysozyme, the forbidden immunogen, could prime H-2^b mice for an augmented proliferative response to ringed-neck pheasant lysozyme, a closely related permitted immunogen. Low responsiveness to variants of lysozyme was found to be related to a suppressor mechanism [14]. Similarly, we have results of preliminary experiments that indicate that H-2^k mice immunized with BoI plus adjuvant have cells that actively suppress a response to the BoI determinant. Therefore, it is conceivable that suppressor cells might be important in selecting antigenic determinants in several cases controlled by MHC Ir genes. However, these putative suppressor cells would have to express affinities that differ from those of effector cells to explain the relationships we observed between forbidden and pemitted variants of the ACL determinant.

It is challenging to attempt to envision the mechanism by which MHC gene products might control differences in affinity between suppressor and effector T lymphocytes.

In a previous report [13], we proposed that selection of determinants by antigen-presenting cells might result from the orientation of the immunogen as it lies at anchor in the membrane of the presenting cell. MHC gene products comprising part of the structure of the antigen-presenting patch of membrane could help define the most stable orientation of the immunogen and hence control which determinants were displayed or buried. Orientation of the immunogen could be influenced by the integrated physicochemical properties of the immunogen itself, as well as of the lipid and MHC glycoprotein components of the antigen-presenting patch of membrane. Therefore, small allelic differences in MHC products, chemical substitutions in the immunogen, or changes in the metabolism of the cell membrane could all contribute to the availability of particular determinants for presentation.

It has been shown that independent populations of T lymphocytes recognize an antigen in association with different MHC gene products in F_1 hybrid animals [15] and probably in homozygous animals as well [16]. It is therefore conceivable that helper and suppressor lymphocytes each could be triggered by insulin associated with different H-2 products. This association could influence the orientation of the molecule and its determinants in such a way that helper and suppressor cells would be exposed to different determinants, or to the same determinant in a different alignment. The different exposures of antigenic determinants could lead to selection of a different spectrum of antigen receptors within each class of cells. In this way, the same immunogen could select helper T cells with antigen receptors of certain affinities and suppressor T cells with receptors of other affinities.

Dynamic interaction between such different populations of suppressor and activator cells might generate the H-2-controlled responses which we have observed including cross-priming and immunodominance. An attractive feature of this model is that it proposes a way for MHC genes to regulate the immune response to defined antigenic determinants without having to assume that MHC genes influence the structure of the antigen receptor of the T lymphocytes. It appears that the idiotypes of T cells [17] and T cell factors [18] are genetically linked not to MHC genes but to immunoglobulin heavy chain allotype markers.

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Qed-1 – a target for unrestricted killing by T cells

Two prototype target determinants for unrestricted killing by T cells are defined: Qed-1^a, detected on B6.Tla^a targets by C3H/HeJ lymphocytes primed *in vivo* and restimulated *in vitro* by B10.BR spleen cells; and Qed-1^b, detected on C57BL/6J lymphocytes by B10.BR anti-C3H/HeJ effector cells generated in the same manner. Other mouse strains can be typed for Qed-1 by the ability of their lymphocytes to inhibit one of these lytic reactions. Of 55 inbred strains, 52 expressed either Qed-1^a or Qed-1^b, which thus behaved as products of alleles of a single locus, Qed-1. The remaining three strains, all H-2^r, did not compete against specific lysis of Qed-1^a, but inhibited Qed-1^b-specific lysis only in part; it is proposed that these strains carry a third allele or haplotype, Qed-1^c. The Qed-1 locus was mapped distal to Qa-2.
Qed-1^b was found on both normal and mitogen-activated lymphocytes and did not appear confined to any lymphoid subpopulation. Cytotoxic responses, not restricted by H-2 and specific for antigens controlled by the Tla region, could be induced in several combinations of H-2-identical strains differing at Qed-1. Cells of some strains, like B10.BR, NZB, and SWR, responded directly in culture, even without priming *in vivo*.

1 Introduction

Antigens encoded by the H-2K, H-2D and H-2L loci of the mouse major histocompatibility complex readily stimulate primary cytotoxic responses *in vitro* and are recognized on target cells independently of other antigens [1, 2]. Minor histocompatibility (H) antigens, on the other hand, set off such responses only after *in vivo* sensitization, and a cell is not killed unless it expresses both the minor H antigen and a restriction element (which may be H-2K, H-2D or H-2L) shared with the stimulator [3].

It has been found recently that after *in vivo* sensitization, antigens controlled by the Tla region give rise to killer cells unrestricted by H-2. Forman and Flaherty [4] raised killer cells between two sublines differing at Qa-2, while Klein and Chiang [5] reported that, in addition to H-2I^k antigens, A.TH anti-A.TL effector cells detect an antigen H-2T controlled by a locus to the right of Qa-2. Kastner and Rich [6] and Fischer Lindahl [7] made similar observations with B10.BR effector cells sensitized against H-2-identical AKR, BALB.C3H, CBA or C3H cells. These effector cells contained a subpopu-

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Abbreviations: C: Complement **Con A:** Concanavalin A **H:** Histocompatibility **LPS:** Lipopolysaccharide **PHA:** Phytohemagglutinin **FCS:** Fetal calf serum **EHAA:** Modified Eagle's-Hanks' amino acid-supplemented (medium)

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