CYTOTOXIC T LYMPHOCYTE NONRESPONSIVENESS TO THE MALE ANTIGEN H-Y IN THE H-2D^b MUTANTS bm13 AND bm14

Complementation of the Response in F_1 Crosses with the I-A^b Mutant bm12 Nonresponder and Failure of B6 or D^b Mutant Mice Tolerant of Each Other to Respond to Allogeneic Male Cells*

BY LEO P. DE WAAL, ROGER W. MELVOLD,‡ AND CORNELIS J. M. MELIEF

From the Department of Tumor Immunology, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands; and ‡Department of Microbiology-Immunology and the Cancer Center, Northwestern University, Chicago, Illinois 60611

Cytotoxic T lymphocyte (CTL)[1] responses to viruses, minor histocompatibility antigens, and haptens are regulated by both class-I and class-II gene products of the major histocompatibility complex (MHC). This two-gene control is best illustrated by the CTL response against the male antigen H-Y. In C57BL/6 (B6, H-2^b) mice generation of H-Y–specific CTL involves the presentation of H-Y by adherent cells in the context of the I-A^b-class-II molecule to T helper cells; and presentation of the antigen by nucleated cells in the context of the H-2D^b class-I molecule to CTL precursors. Accordingly, anti-H-Y B6 T helper cells are I-A^b-restricted and anti-H-Y B6 CTL are H-2 D^b-restricted (1–3).

Class-I molecules function as immune-response (Ir) gene products regulating CTL responses against a variety of antigens (1, 2, 4–7). An opportunity to more precisely analyze this immunoregulatory function is provided by the availability of H-2 mutants. The H-2 K^b mutants have been used intensively in this respect, and they have profound effects on the magnitude and specificity of H-2 K-restricted CTL responses. The pattern of cross-reactivity of antigen-specific B6 CTL tested on a panel of antigen-bearing H-2 K^b mutant target cells is quite similar for the CTL responses against Ectromelia virus, LCM virus, Vaccinia virus, SV 40, Sendai virus, H-4 and H-3 minor histocompatibility antigens, and the hapten AED (8–15). In these antigenic systems, the K^b mutant target cells to a greater or lesser extent show loss of K^b-restriction sites. Subtle differences

* Supported in part by the Foundation for Medical Research, FUNGO, which is subsidized by The Netherlands Organization for the Advancement of Pure Research (ZWO).

‡ Correspondence should be addressed to L. P. de Waal, % Publication Secretariat, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, P. O. Box 9406, 1006 AD Amsterdam, The Netherlands. No reprints are available.

[1] *Abbreviations used in this paper:* apc, antigen-presenting cell; CTL, cytotoxic T lymphocyte; IMDM, Iscove's modified Dulbecco's medium; Ir, immune response; LPS, lipopolysaccharide; MHC, major histocompatibility complex.

between the various antigenic systems occur, indicating that different restriction sites on the H-2 K^b molecule are used for different antigens. Besides CTL restriction specificities, the mutations also affect the capacity to respond. B6 and H-2 K^b mutants vary in their ability to generate H-2-restricted CTL responses against SV 40, Sendai virus (12, 13) H-4 and H-3 minor histocompatibility antigens (16) and VSV (Forman, personal communication). In all three systems, the bml mutant shows Ir defects and does not mount an H-2K-restricted CTL response. The mechanism of this type of Ir-gene-controlled nonresponsiveness is not known.

Similar to the situation with the H-2 K^b mutants, evidence was presented that two H-2 D^b mutations, bm13 and bm14, strongly influenced the H-2 D^b-restricted CTL responses against the hapten AED and against Moloney leukemia virus (15, 17). We now report the effect of these mutations on the H-2 D-restricted CTL response against H-Y.

The results indicate unequivocally for the first time with H-2 mutants that class I and class II MHC-determined CTL response defects complement each other in an F_1 hybrid animal. Moreover, in this model evidence is presented that Ir genes may act at the level of both the T cell repertoire and antigen presentation.

Materials and Methods

Animals. All mice were bred at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service. The mutant strains B6.C-H-2^{bm13} (bm13) and B6.C-H-2^{bm14} (bm14) (18) were raised from breeding stocks supplied by Dr. R. W. Melvold and Dr. D. W. Bailey (The Jackson Laboratory, Bar Harbor, ME), respectively.

Immunization. Unless otherwise mentioned, female mice were primed by one intraperitoneal (i.p.) injection of 1.5×10^7 male spleen cells in 0.5 ml phosphate-buffered saline (PBS).

Neonatal Tolerance Induction. Female bm14 or B6 mice were injected intraperitoneally within 24 h after birth with a mixture of 5×10^7 spleen and bone marrow cells from female (B6 × bm14)F_1 animals in a volume of 0.1 ml. Tolerance was tested by skin grafting.

In Vitro Generation of H-Y–Specific CTL. CTL were generated as previously described (3). Spleen cells (10^8) from in vivo–primed female mice were stimulated with 2,500 rad-irradiated male spleen cells (10^8) in 80-ml culture medium for 5 d at 37°C in humidified air with 5% CO_2. The culture medium consisted of Iscove's modified Dulbecco's medium (IMDM; Gibco Laboratories, Grand Island, NY) supplemented with 10% pooled human serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), and 2-mercaptoethanol (2×10^{-5} M).

Cell-mediated Lymphocytotoxicity. Varying numbers of effector cells were added to 3×10^4 $Na_2{}^{51}CrO_4$ (^{51}Cr)-labeled target cells in 0.2 ml IMDM supplemented with 10% fetal calf serum (Gibco Laboratories) in wells of round-bottom microtiter plates and incubated for 3 h at 37°C in humidified air with 5% CO_2. LPS- (30 μg/ml) induced lymphoblasts were used as target cells. After incubation, the supernatant was collected with the Titertek Supernatant Collection System (Flow Laboratories, Inc., Mclean, VA). The percentage specific ^{51}Cr release was calculated by the formula:

$$\% \text{ specific lysis} = \frac{\text{cpm experimental well-background } ^{51}\text{Cr-release}}{\text{cpm 5\% saponin release-background } ^{51}\text{Cr-release}} \times 100.$$

The standard error of triplicate cultures was always less than 3% specific ^{51}Cr release.

Results

bm13 and bm14 Mice Are Low Responders in the CTL Response to H-Y. H-Y-primed bm13 and bm14 spleen cells restimulated in vitro with male syngeneic spleen cells did not generate H-Y–specific CTL (Table I). However, in a small number of experiments (~1 out of 10) we observed weak H-Y–specific CTL responses in these mutants, indicating that bm13 and bm14 are not complete CTL nonresponders to H-Y (data not shown). Different immunization procedures (s.c.; i.v.; footpad immunization) or the addition of IL-2 to the culture medium during in vitro restimulation did not elevate the low CTL responsiveness of bm13 and bm14 to H-Y (data not shown). In contrast, H-Y–primed B6 spleen cells showed strong H-Y–specific CTL responses restricted by H-2 D^b (Table I). Furthermore, B6 anti–H-Y CTL were not able to lyse male bm13 or male bm14 target cells (Table I). This indicates that the mutations in bm13 and bm14 have altered extensively the B6 H-Y–specific restriction specificities. Allogeneic CTL specific for the mutated H-2 D^b molecule could efficiently lyse bm13 and bm14 target cells, indicating that defective expression of the H-2 D molecule on the target cells cannot explain the above findings (Table II).

Fine Specificity of Alloimmune CTL Directed Against H-2 D. Effector cells were generated in all possible combinations between B6, bm13, and bm14 and tested against B6, bm13, and bm14 target cells. The results in Table II indicate that bm13 is closer to B6 than is bm14 with respect to CTL allospecificities. B6 responder cells generated a weak CTL response against bm13 alloantigen, in contrast to the strong response observed against bm14. Furthermore, bm14 anti-bm13 CTL lysed B6 target cells to the same extent as bm13 targets, whereas bm13 anti-bm14 CTL showed a much lower level of cross-reactivity against B6 targets. Another interesting observation is the asymmetry in CTL responses. In contrast to the weak B6 anti-bm13 CTL response, bm13 responder cells generate strong alloreactivity against B6. bm13 anti-B6 CTL and bm14 anti-B6 CTL crosskill, to some extent, bm14 and bm13 targets, respectively. However, in the reverse direction, B6 anti-bm13 and B6 anti-bm14 CTL do not show any cross-reactivity. Finally, it can be concluded from Table II that both bm13 and bm14 (although the latter to a lesser extent) share determinants with B6, as defined by allogeneic CTL.

TABLE I

H-Y–Specific CTL Response in B6, bm13, and bm14 Mice

CTL anti-H-Y*	Target cells‡					
	B6♂	4R§♂ (k/b)	5R§♂ (b/d)	bm13♂	bm14♂	B6♀
B6	‖63 (9.7)	61 (13.6)	7 (4.3)	5 (3.8)	5 (4.7)	4 (3.1)
bm13	3 (2.4)	3 (3.1)	4 (3.8)	3 (3.4)	4 (3.0)	3 (1.2)
bm14	4 (4.8)	3 (1.8)	3 (3.5)	2 (2.4)	10 (4.5)	1 (2.2)

* Female responder mice were primed in vivo and their spleen cells restimulated in vitro with male syngeneic spleen cells.

‡ LPS blasts, H-2 K and -D alleles in parentheses.

§ 4R = B10.A(4R); 5R = B10.A(5R).

‖ Mean percentage specific cytotoxicity of eight experiments; SE in parentheses; effector-to-target ratio 16:1.

TABLE II

Fine Specificity of Alloimmune Cytotoxic T Lymphocytes Directed Against H-2 D

Allo-CTL*	Target cells‡		
	bm13	bm14	B6
B6 anti-bm13	16§	1	0
B6 anti-bm14	2	69	4
bm13 anti-B6	3	27	55
bm14 anti-B6	26	4	52
bm13 anti-bm14	5	70	33
bm14 anti-bm13	57	3	56

* 5×10^7 responder cells were cultured with 5×10^7 stimulator cells (2,500 rad irr.) in 80 ml culture medium during 5 d at 37°C, after which period the effector cells were tested on ^{51}Cr-labeled target cells.

‡ LPS blasts.

§ Mean percentage specific ^{51}Cr-release of three experiments; effector to target cell ratio 16:1.

(B6 × bm13)F_1 and (B6 × bm14)F_1 Hybrids Respond to H-Y After Sensitization with Male F_1 or Male B6 Cells But Not with Mutant Male Cells. (B6 × bm13)F_1 and (B6 × bm14)F_1 females, primed in vivo and restimulated in vitro with syngeneic F_1 male spleen cells, generated efficient H-Y–specific CTL responses. Like B6 anti–H-Y CTL, these F_1 effectors preferentially lyse male target cells, expressing the H-2 D^b-gene product: male B10.A(4R) (K^kD^b) target cells were lysed, whereas male B10.A(5R) (K^bD^d) target cells were not lysed (Table III *A*). After sensitization of F_1 females with male B6 spleen cell, identical results were obtained (Table III *A*). However, sensitization of (B6 × bm13)F_1 females with male bm13 spleen cells or sensitization of (B6 × bm14)F_1 females with male bm14 spleen cells did not result in the generation of H-Y–specific CTL (Table III *A*). Finally, (B6 × bm13)F_1 females were primed in vivo by either F_1 or B6 male spleen cells and restimulated in vitro with male bm13 spleen cells and vice versa. Also under these conditions we could not measure any H-Y–specific CTL activity (Table III *B*).

Genetic Complementation Between bm12, bm13, and bm14 for H-Y–Specific CTL Responses. The I-A^b mutant bm12 is a nonresponder in CTL reactivity and T cell proliferation to H-Y (3, 19). Therefore, it was of interest to see whether bm12 could complement bm13 or bm14 in an F_1 hybrid for the H-Y–specific CTL response. From Table IV, it is clear that (bm12 × bm13)F_1 and (bm12 × bm14)F_1 hybrids can respond against H-Y, indicating transcomplementation between a class-II molecule (I-A^b) involved in the antigen-specific recognition by T helper cells and a class I molecule (H-2 D^b) involved in the antigen-specific recognition by CTL precursors. As expected, bm13 and bm14 could not complement each other for the generation of H-Y–specific CTL (Table IV).

Failure of B6 or D^b Mutant Mice Tolerant of Each Other to Respond to Tolerogenic Allogeneic Male Cells. The mechanism underlying the inability of bm14 to generate anti-H-Y CTL was further investigated by injecting female bm14 mice within 24 h after birth with a mixture of spleen and bone-marrow cells of (B6 × bm14)F_1 female mice. These neonatally injected bm14 mice were tested after 8

TABLE III

H-Y–Specific CTL Response of (B6 × bm13)F_1 and (B6 × bm14)F_1 Mice

A. Responder mice	Sensitization to H-Y with*	Target cells‡			
		F_1§♂	4R‖♂ (k/b)	5R‖♂ (b/d)	F_1§♀
(B6 × bm13)F_1♀	(B6 × bm13)F_1♂	39¶	46	−2	4
	B6♂	45	47	5	4
	bm13♂	4	3	1	3
(B6 × bm14)F_1♀	(B6 × bm14)F_1♂	40	NT	NT	1
	B6♂	42	NT	NT	2
	bm14♂	3	−1	2	2
B. Responder mice*	Primed in vivo with	Restimulated in vitro with		Target cells§	
				B6♂	B6♀
(B6 × bm13)F_1♀	(B6 × bm13)F_1♂	(B6 × bm13)F_1♂		68¶	4
	(B6 × bm13)F_1♂	bm13♂			
	bm13♂	(B6 × bm13)F_1♂		2	−1
				−2	3

* Responder female mice were primed in vivo and their spleen cells restimulated in vitro with the indicated male spleen cells.
‡ LPS blasts; H-2 K and -D alleles in parentheses.
§ F_1 hybrid target cells syngeneic with the responder haplotype were used.
‖ 4R = B10.A(4R); 5R = B10.A(5R).
¶ Percentage specific ^{51}Cr-release; effector to target cell ratio 16:1.
NT, not tested.

TABLE IV

Genetic Complementation of the H-Y–Specific CTL Response

Responder mice	Sensitization to H-Y with*	Target cells‡			
		B6♂	B6♀	(bm13 × bm14)F_1♂	(bm13 × bm14)F_1♀
(bm12 × bm14)F_1♀	(bm12 × bm14)F_1♂	45§	4		
(bm12 × bm13)F_1♀	(bm12 × bm13)F_1♂	46	6		
(bm13 × bm14)F_1♀	(bm13 × bm14)F_1♂			5	2

* Responder female mice were primed in vivo and their spleen cells restimulated in vitro with the indicated male spleen cells.
‡ LPS blasts.
§ Percentage specific ^{51}Cr-release; effector to target cell ratio 16:1.

wk for tolerance to B6 alloantigens and for their capacity to generate H-Y–specific CTL responses. Table IV indicates that (*a*) bm14 mice can mount efficient CTL responses to B6 alloantigens, (*b*) female bm14 mice neonatally injected with (B6 × bm14)F_1 female cells are tolerant to B6 alloantigens, (*c*) these tolerant mice can generate excellent CTL responses to third-party alloantigens (B10.D2), and (*d*) bm14 female cells tolerant to B6 female alloantigens do not mount an H-Y–specific CTL response after priming in vivo and restimulation in vitro with (B6 × bm14)F_1 male cells. Likewise, female B6 mice were rendered neonatally tolerant for bm14 alloantigens. These tolerant B6 female mice generated excellent H-Y–specific CTL responses after sensitization with male B6 or

TABLE V

Failure of B6 or D^b Mutant Mice Tolerant of Each Other to Respond to Allogeneic Male Cells

Responder mice	Priming in vivo and restimulation in vitro with	Target cells (LPS blasts)		
		P815 (H-2^d)	(B6 × bm14)F_1 ♂	(B6 × bm14)F_1 ♀
bm14♀	(B6 × bm14)F_1♂		51*	44
bm14♀ tol.B6♀	(B6 × bm14)F_1♂		7	1
bm14♀ tol.B6♀	B10.D2 ‡♀			
	B10.D2 ‡♀	83		1
B6♀ tol.bm14♀	B6♂		43	3
B6♀ tol.bm14♀	bm14♂		0	3
B6♀ tol.bm14♀	(B6 × bm14)F_1♂		45	1

* Percentage specific cytotoxicity; effector to target ratio 16:1.

‡ Tolerant bm14 ♀mice were primed in vivo with (B6 × bm14)F_1♂ and restimulated in vitro with either F_1♂ cells or B10.D2 ♀ cells.

male (B6 × bm14)F_1 spleen cells, but failed to respond after sensitization with male bm14 spleen cells. Thus, H-Y–specific CTL responses were only observed in neonatally tolerized animals when responder type T cells and responder type antigen-presenting cells (apc) were used (Table V).

Discussion

Our findings clearly demonstrate that the CTL response to H-Y is regulated by H-2 D gene products. Structural alterations of the H-2 D^b molecule in the bm13 and bm14 mutant strains lead to changes in restriction specificities and in responsiveness. This is further support for the notion that class-I determinants, recognized by H-2 K/D-restricted CTL, act as Ir-gene products (1–7, 13, 16, 17). Changes in restriction specificities in bm13 and bm14 compared with B6 have been observed in three other systems: (*a*) H-2 D^b-restricted Moloney virus-specific CTL do not lyse virus-infected bm13 or bm14 target cells (17); (*b*) H-2 D^b-restricted CTL specific for the hapten AED are unable to kill hapten-modified bm13 and bm14 targets (15), and (*c*) Melvold et al. (20) reported that the biologic activity of a mutant non-H-2 histocompatibility antigen was dependent on the simultaneous presence of both the mutant minor H antigen and H-2 D^b. The bm13 mutant could replace H-2 D^b in this latter interaction, but bm14 could not. This corresponds with our data that bm13 is closer to B6 than bm14 with respect to allogeneic CTL determinants (Table II). The results in Table II also show that bm13 and bm14 still share CTL allodeterminants with B6, which is apparently not reflected in the presence of shared restriction specificities for H-2 D^b-restricted CTL responses to H-Y, Moloney virus, or AED. It can be envisaged, however, that different antigens (like the minor histocompatibility antigen reported above) use different restriction sites on the H-2 D^b molecule. Apart from restriction specificities, the bm13 and bm14 mutations also affect the magnitude of CTL responses: both mutants are CTL nonresponders to H-Y (this paper) and bm14 does not mount a CTL response to Moloney virus (17). However, bm13 does respond to Moloney virus, although the restriction preference is shifted to H-2 K^b (17). Thus, although a close relationship exists

between CTL-restriction specificities, CTL regulating determinants and CTL-allospecificities, in some instances CTL regulating determinants on one class I molecule influence the CTL response restricted by another class I molecule (17, 21).

Our findings are somewhat in contrast with data of Simpson et al. (22) who showed that bm14 can generate H-Y–specific CTL, whereas we observed weak H-Y–specific CTL responses only in a minority of experiments.

From our analysis of the CTL nonresponsiveness to H-Y of the bm12 I-A mutant, we concluded that T helper cells recognize the H-Y antigen in the context of the I-$A^b\alpha\beta$ (class-II) molecule on adherent cells, whereas CTL precursors recognize the antigen in the context of the D^b (class-I) molecule on adherent as well as nonadherent cells. With spleen cells from primed animals, the requirement for T helper cells could be bypassed with interleukin-2 (3). The current finding that the bm12 class-II mutant on the one hand and the bm13 and bm14 class-I mutants on the other complement each other for the H-Y–specific CTL response, further supports the idea that two separate Ir gene–controlled pathways are needed for this response (2). Moreover, the complementation data show that the need for tolerance of the mutant class-II and class-I molecules in the (bm12 × bm13)F_1 and (bm12 × bm14)F_1 hybrids does not create deletions in the I-A^b-restricted T helper cell and D^b-restricted CTL repertoire for H-Y. The same holds for the D^b-restricted CTL repertoire for H-Y in the (B6 × bm13)F_1 and (B6 × bm14)F_1 hybrids.

The two main observations pertinent to the mechanism of H-2 D-controlled nonresponsiveness are the following: *1*) (responder × nonresponder)F_1 cells react against antigen presented on F_1 or responder parental cells but do not respond to antigen presented on nonresponder parental cells; *2*) B6 or D^b mutant mice tolerant of each other fail to respond to the male antigen presented on the tolerogenic allogeneic cell. Observation *1* is the classical response pattern in Ir gene–controlled nonresponsiveness, although we recently documented an important exception to this rule in experiments on the nonresponsiveness to H-Y of the bm12 I-A mutant (3). The classical response pattern can be explained along two main lines: lack of appropriate antigen presentation, or deletion(s) in the T cell repertoire (23–25). Observation *2*) is in line with other recent experiments demonstrating holes in the allorestricted T cell repertoire. Thus, it was shown that B6 mice neonatally tolerant of $H\text{-}2^k$ did not generate a CTL response to $H\text{-}2^k$ plus vaccinia virus (26), although B6 spleen cells acutely depleted of anti-$H\text{-}2^k$ reactivity by filtration through semiallogeneic recombinant or F_1 hosts did respond to $H\text{-}2^k$ plus vaccinia virus (26). In the proliferative T cell response against the synthetic polypeptide GLT, it was seen that elimination by BUdR and light of T cells specific for alloantigen X can create a deletion in the T cell repertoire restricted by alloantigen Y (27).

In contrast to our experiments concerning allorestricted CTL responses in a responder/nonresponder neonatal tolerance situation, excellent allorestricted T cell populations specific for minor histocompatibility antigens and the hapten TNP were seen following neonatal tolerance induction using two responder type strains (28, 29). H-2 Ir gene–controlled nonresponsiveness is currently attributed to the absence of functional T cells rather than inappropriate antigen presenta-

tion (30–33), the evidence being mainly based on high responses of high-responder type T cells to antigen presented on low-responder type antigen-presenting cells (apc).

However, the situation may not be as simple as that, because (*a*) the antigen seen on apc in the context of allogeneic MHC molecules is different from that seen on syngeneic apc and may therefore be under a different Ir gene control as in fact demonstrated (30) (*b*). Cytochrome c–specific T cell hybridomas of B10.A and B10.A(5R) origin only responded to pigeon cytochrome *c* fragment 81-104 when this antigen was presented on high-responder B10.A apc, but not when presented on low-responder B10.A(5R) apc. This indicates that the relevant MHC molecule (Ia in this case) on the apc determined whether these hybridomas were triggered (34). In later experiments, these results were extended to the population level (35). Therefore, in the final analysis the various interactions between antigen, MHC molecules, and T cell receptors probably determine whether a reaction is triggered. This notion does not rule out that nonresponsiveness may be the result of lack of T cells in the repertoire that are capable of being triggered by a particular antigen-MHC combination. For example, in our analysis of the CTL nonresponsiveness to H-Y of the bm12 I-A mutant, only evidence for a deletion in the T cells was found. In the neonatal tolerance experiments with the bm13 and bm14 nonresponders to H-Y, a response was only seen with responder type T cells and responder type apc, indicating that both the T cell source and the MHC type of the apc have to be taken into account in this experimental situation.

Summary

The cytotoxic T-lymphocyte (CTL) response against the male-specific antigen H-Y in C57BL/6 (B6, H-2^b) mice is regulated by the I-A^b and D^b molecules. From previous studies, we concluded that the bm12 I-A^b mutant does not respond to H-Y, because of a deletion in its T-helper-cell repertoire. We now demonstrate that two D^b mutants, bm13 and bm14, also fail to generate a CTL response to H-Y. The bm12 class-II mutant on one hand and the bm13 and bm14 class-I mutants on the other complemented each other for the H-Y–specific CTL response in (bm12 × bm13)F_1 and (bm12 × bm14)F_1 hybrids. This indicates that the need for tolerance of the mutant class II and class I molecules in these hybrids does not create deletions in the I-A^b–restricted T helper cell and D^b-restricted CTL repertoire for H-Y. This study constitutes the first demonstration with H-2 mutants that a CTL response controlled by class I and class II MHC molecules is complemented in an F_1 cross between a class I and a class II nonresponder. (B6 × bm13)F_1 and (B6 × bm14)F_1 hybrids only responded to H-Y when the antigen was presented on F_1 or B6 antigen-presenting cells (apc) but not on D^b mutant apc. B6 or D^b mutant responders rendered neonatally tolerant of each other failed to respond to the H-Y antigen presented on the tolerogenic allogeneic cell. In the tolerized animals, a response was only seen with responder (B6) type T cells and responder type (B6) apc, indicating that both the T cell source and the MHC type of the apc have to be taken into account in this system. Thus, Ir genes may act at the level of both the T cell repertoire and antigen presentation.

Received for publication 31 May 1983.

References

1. Von Boehmer, H., and W. Haas. 1979. Distinct Ir genes for helper and killer cells in the cytotoxic response to H-Y antigen. *J. Exp. Med.* 150:1134.
2. Simpson, E. 1982. The role of H-Y as a minor transplantation antigen. *Immunology Today.* 3:97.
3. de Waal, L. P., J. de Hoop, M. J. Stukart, H. Gleichmann, R. W. Melvold, and C. J. M. Melief. 1983. Nonresponsiveness to the male antigen H-Y in H-2 I-A mutant B6.C-H-2^{bm12} is not caused by defective antigen presentation. *J. Immunol.* 130:665.
4. Doherty, P. C., W. E. Biddison, J. R. Bennink, and B. B.Knowles. 1978. Cytotoxic T cell response in mice infected with influenza and vaccinia viruses vary in magnitude with H-2 genotype. *J. Exp. Med.* 148:534.
5. Zinkernagel, R. M., A. Althage, S. Cooper, G. Kreel, P. A. Klein, B. Sefton, L. Flaherty, J. Stimpfling, D. Shreffler, and J. Klein. 1978. Ir genes in H-2 regulate generation of antiviral cytotoxic T cells. Mapping to K or D and dominance of unresponsiveness. *J. Exp. Med.* 148:592.
6. Wettstein, P. J., and J. A. Frelinger. 1980. H-2 effects on cell-cell interactions in the response to single non-H-2 alloantigens. III. Evidence for a second Ir gene system in the H-2 K and H-2 D regions. *Immunogenetics.* 10:211.
7. Rosenthal, K. L., and R. M. Zinkernagel. 1981. Inability of mice to generate cytotoxic T lymphocytes to vesicular stomatitis virus restricted to H-2 K^k or H-2 D^k. *J. Immunol.* 126:446.
8. Blanden, R. V., M. B. C. Dunlop, P. C. Doherty, H. I. Kohn, and I. F. C. McKenzie. 1976. Effects of four H-2 K mutations on virus-induced antigens recognized by cytotoxic T cells. *Immunogenetics* 3:541.
9. Zinkernagel, R. M. 1976. H-2 compatibility requirement for virus-specific T cell–mediated cytolysis. *J. Exp. Med.* 143:437.
10. Zinkernagel, R. M., and J. Klein. 1977. H-2 associated specificity of virus immune cytotoxic T cells from H-2 mutant and wild mice: M523 (H-2 K^{ka}) and M505 (H-2 K^{bd}) do, M504 (H-2 D^{da}) M506 (H-2 K^{fa}) do not cross react with wild type H-2 K or H-2 D. *Immunogenetics.* 4:581.
11. Hurwitz, J. L., S. Pan, P. J. Wettstein, and P. C. Doherty. 1983. Cross-reactivity patterns of vaccinia specific cytotoxic T-lymphocytes from H-2K^b-mutants. *Immunogenetics.* 17:79
12. Pan, S., P. J. Wettstein, and B. B. Knowles. 1982. H-2 K^b mutations limit the cytotoxic T-lymphocyte response to SV40 TASA. *J. Immunol.* 128:243.
13. de Waal, L. P., W. M. Kast, R. W. Melvold, and C. J. M. Melief. 1983. Regulation of the cytotoxic T-lymphocyte response against Sendai virus analyzed with H-2 mutants. *J. Immunol.* 130:1090.
14. Wettstein, P. J. 1982. H-2 effects on cell-cell interactions in the response to single non-H-2 alloantigens. Effect of H-2 K^b mutations on presentation of H-4 and H-3 alloantigens. *J. Immunol.* 128:2629.
15. Levy, R. B., and G. M. Shearer. 1982. Cell-mediated lympholysis response against autologous cells modified with haptenic sulfhydryl reagents. *J. Immunol.* 129:1525.
16. Wettstein, P. J., and R. W. Melvold. 1983. H-2 effects on cell-cell interactions in the response to single non-H-2 antigens. *Immunogenetics.* 17:109.
17. Stukart, M. J., A. Vos, J. Boes, R. W. Melvold, D. W. Bailey, and C. J. M. Melief. 1982. A crucial role of the H-2 D locus in the regulation of both the D and K-associated cytotoxic T-lymphocyte response against Moloney virus, demonstrated with two D^b mutants. *J. Immunol.* 128:1360.

18. Morgan, G. M., H. Dellos, I. F. C. McKenzie, R. W. Melvold, and D. W. Bailey. 1980. Studies of two H-2 D^b mutants: B6.C-H-2^{bm13} and B6.C-H-2^{bm14}. *Immunogenetics.* 11:341.
19. Michaelides, M., M. Sandrin, G. Morgan, I. F. C. McKenzie, R. Asham, and R. W. Melvold. 1981. Ir gene function in an I-A subregion mutant B6.C-H-2^{bm12}. *J. Exp. Med.* 153:44.
20. Melvold, R. W., H. I. Kohn, and D. W. Bailey. 1980. Interaction of H-2 D^b with mutant histocompatibility gene H (KH-11) in the mouse. *Immunogenetics.* 11:597.
21. Reiss, C. S., M. E. Dorf, B. Benacerraf, and S. J. Burakoff. 1982. Genetic control of the cytotoxic T-lymphocyte response to influenza viruses: H-2 D genes influence the response to H-2 K^b plus virus. *J. Immunol.* 128:2295
22. Simpson, E., R. D. Gordon, P. R. Chandler, and D. Bailey. 1978. Anti-H-Y responses of H-2^b mutant mice. *Eur. J. Immunol.* 8:685.
23. Nagy, Z. A., C. N. Baxevanis, N. Ishii, and J. Klein. 1981. Ia antigens as restriction molecules in Ir gene controlled T-cell proliferation. *Immunol. Rev.* 60:59.
24. Shevach, E. 1982. Is determinant selection dead? *Immunology Today.* 3:31.
25. Rosenthal, A. S. 1982. Determinant selection and macrophage function. *Immunology Today.* 3:33.
26. Schwartz, D. H., and P. C. Doherty. 1982. Induction of neonatal tolerance to H-2^k in B6 mice does not allow the emergence of T cells specific for H-2^k plus vaccinia virus. *J. Exp. Med.* 156:810.
27. Ishii, N., Z. A. Nagy, and J. Klein. 1983. In vitro correlate for a clonal deletion mechanism of immune response gene controlled nonresponsiveness. *J. Exp. Med.* 157:998.
28. Forman, J., J. Klein, and J. W. Streilein. 1977. Spleen cells from animals neonatally tolerant to H-2 K^k antigens recognize trinitrophenyl-modified H-2 K^k spleen cells. *Immunogenetics.* 5:561.
29. Forman, J., and J. W. Streilein. 1979. T cells recognize minor histocompatibility antigens on H-2 allogeneic cells. *J. Exp. Med.* 150:1001.
30. Ishii, N., Z. A. Nagy, and J. Klein. 1982. Absence of Ir gene control of T cells recognizing foreign antigen in the context of allogeneic MHC molecules. *Nature (Lond.).* 295:531.
31. Kimoto, M., T. J. Krenz, and C. G. Fathman. 1981. Antigen reactive T cell clones. III. Low responder antigen-presenting cells function effectively to present to selected T-cell clones derived from (high responder × low responder)F_1 mice. *J. Exp. Med.* 154:883.
32. Clark, R. B., and E. M. Shevach. 1982. Generation of T cell colonies from responder strain 2 guinea pigs that recognize the copolymer L-glutamic acid, L-lysine in association with nonresponder strain 13 Ia antigens. *J. Exp. Med.* 155:635.
33. Thomas, D. W., and M. D. Hoffman. 1982. Lack of macrophage defect in presentation of antigens under Ir gene control. *J. Immunol.* 128:780.
34. Heber-Katz, E., R. H. Schwartz, L. A. Matis, C. Hannum, T. Fairwell, E. Appella, and D. Hansburg. 1982. Contribution of antigen-presenting cell major histocompatibility complex gene products to the specificity of antigen-induced T cell activation. *J. Exp. Med.* 155:1086.
35. Heber-Katz, E., D. Hansburg, and R. H. Schwartz. 1983. The Ia molecule of the antigen-presenting cell plays a critical role in immune response gene regulation of T cell activation. *J. Mol. Cell. Immunol.* in press.