

**H-2 COMPATIBILITY REQUIREMENT FOR VIRUS-SPECIFIC
T-CELL-MEDIATED CYTOLYSIS**

**The *H-2K* Structure Involved is Coded by a Single Cistron
Defined by *H-2K^b* Mutant Mice***

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Virus-specific cytotoxic T cells generated in mice infected with lymphocytic choriomeningitis (LCM) virus (1-3) or pox viruses (3) lyse infected target cells only when both are compatible at *H-2K* or *H-2D* of the *H-2* gene region. Also, cytotoxicity of T cells against TNP-modified spleen cells (4, 5), or against minor histocompatibility antigens (6) or the male Y antigen (7) is apparently similarly restricted.

A variety of mouse strains derived from C57BL/6 (B6) [e.g., B6.Hz1(*H-2^{ba}*) and B6.Hz170(*H-2^{bf}*)] express a mutation in the *K* region of the *H-2* gene complex which, when tested against wild-type B6, causes mutual rejection of skin grafts in vivo (8) and positive mixed lymphocyte reactions and generation of cytotoxic T cells in vitro (reference 9; and D. W. Bailey, personal communication). The mutation is not detected serologically (9, 10). B6 *H-2^{ba}* and B6 *H-2^{bf}* differ from B6 probably in a single allelic point mutation in the *K* region of the *H-2* gene complex, affecting the same locus as shown by complementation studies (D. W. Bailey, personal communication; and reference 11).

The availability of these *H-2* mutant mice offered a possibility to analyze further the genetic requirements for the lytic interaction of virus-specific cytotoxic T cells and target cells. The results indicate that the same cistron which codes for the structure recognized by alloantigen-reactive T cells and which is defined by the B6 *H-2^{ba}* and *H-2^{bf}* mutant mice is also central to the apparent restriction of virus-specific cytotoxic T cells by the *H-2* gene complex.

Materials and Methods

Mice. 6- to 9-wk-old C57BL/10 (B10), B10.A(2r), B10.A(5r), B6Hz1(*H-2^{ba}*), and B6.Hz170(*H-2^{bf}*) mice were from The Jackson Laboratory, Bar Harbor, Maine. The *H-2* haplotypes are indicated in Tables I and II.

Virus and Immunization. The WE strain of LCM virus was obtained from Doctors M. B. A. Oldstone and R. Welsh, Scripps Clinic and Research Foundation, La Jolla, Calif., and grown in L

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TABLE I
LCM Virus-Specific Cytotoxic Activity of Wild Type and H-2K Mutant H-2^b Mice

Mouse strain H-2 haplotype‡ K $\frac{1}{ABC}$ SD	Spleen cells	⁵¹ Cr release from target macrophages*							
		B10.A(5r)		B/6 H-2 ^{ba}		B/6 H-2 ^{bf}		B10	
		In- fected	Normal	In- fected	Normal	In- fected	Normal	In- fected	Normal
B/6 H-2 ^{ba} ba bbb b b	Immune	44.9	50.6§	94.9	40.4	48.0	33.6	53.4	32.5
	Normal	40.7	40.8	45.9	38.5	30.8	29.8	32.1	30.3
B/6 H-2 ^{bf} bf bbb b b	Immune	54.8	51.7	97.3	45.8	67.3	32.4	73.0	31.8
	Normal	38.9	40.1	43.9	35.8	29.7	32.6	29.9	31.3
B10 b bbb b b	Immune	69.8	45.7	89.3	38.7	61.0	31.2	60.9	30.1
	Normal	38.9	40.1	43.9	35.8	29.7	32.6	29.9	31.3
B10.A(5r) b bbd d d	Immune	74.5	40.8	40.8	35.3	36.7	32.7	52.7	28.2
	Normal	38.9	40.1	43.9	35.8	29.7	32.6	29.9	31.3

* 2×10^6 spleen cells were assayed on 1×10^5 target cells for 12 h at 37°C.

‡ From Shreffler and David (10).

§ Means of triplicates; the SEM were between 2.5 and 5.1.

|| Significantly ($P < 0.05$) different from immune spleen cells on infected and normal spleen cells on infected or normal target cells (where applicable).

cells. Mice were injected intravenously (i.v.) with 5×10^3 plaque-forming units (PFU) and spleens were harvested 7 days later (2). Purified vaccinia virus (WR strains) was a gift from Dr. W. K. Joklik, Duke University, Durham, N. C. Mice were injected intraperitoneally (i.p.) with 2×10^7 PFU and spleen cells were harvested 6 days later (12, 13).

⁵¹Cr-Release Assay. The techniques employed have been described (12, 14) with the only modification being that macrophages were harvested from ether-anesthetized mice injected i.p. 5 days previously with 1 ml of thioglycollate medium (Baltimore Biological Laboratories, Cockeysville, Md.) generously donated by Dr. W. Boe (15). In a preliminary test it was shown that unstimulated macrophages and thioglycollate-induced macrophages were comparable as targets.

Briefly, macrophages were plated into flat bottom 96-hole hemagglutination trays (Falcon Plastics, Div. of BioQuest., Oxnard, Calif.) at 2×10^5 cells per well. After 24 h, part of the resulting monolayers were infected with LCM virus for 24 h and then labeled with ⁵¹Cr; other monolayers were labeled with ⁵¹Cr before infection with vaccinia virus at a 10:1 multiplicity for 5 h (12). Cold target competition experiments were performed as described (16). Competitor cells were infected for 5 h directly after harvest and kept from adhering on a sample mixer machine. ⁵¹Cr release was determined after 5–12 h; water release represents 100% and results are not corrected for spontaneous release. Means \pm SEM of triplicates were determined and compared by using the Student's *t* test.

Results

LCM and vaccinia virus immune spleen cells of wild-type B6 mice, mutants in H-2K^b, and H-2 recombinant mice were reciprocally assayed for virus-specific cytotoxicity on macrophage target cells (Tables I and II). Lysis was recorded as significant only when immune spleen cells on virus-infected targets caused significantly greater ⁵¹Cr release than immune cells on uninfected targets and normal cells on infected targets. No significant lysis was observed when immune B6 H-2^{ba} and B6 H-2^{bf} were assayed on infected B10.A(5r) targets and vice versa, yet all these cell populations were cytotoxic for autochthonous targets and other H-2K or H-2D compatible targets. Preliminary results independently obtained with ectromelia and LCM with H-2^{ba} mice are compatible with these results (R. V. Blanden and P. C. Doherty, personal communication). Although

TABLE II
Vaccinia Virus-Specific Cytotoxic Activity of Wild Type and H-2K Mutant H-2^b Mice

Mouse strain <i>H-2</i> haplotype K $\frac{1}{ABC}$ SD	Spleen cells	⁵¹ Cr release from target macrophages*									
		B10.A(5r)		B10.A(2r)		B/6 <i>H-2^{ba}</i>		B/6 <i>H-2^{bf}</i>		B10	
		In- fected	Nor- mal	In- fected	Nor- mal	In- fected	Nor- mal	In- fected	Nor- mal	In- fected	Nor- mal
B6 <i>H-2^{ba}</i> <i>ba bbb b b</i>	Immune†	26.8§	27.0	81.8	19.2	85.8	30.2	49.8	22.8	58.0	18.9
	Normal	22.7	—	22.8	19.8	28.9	26.8	21.2	23.2	22.1	17.6
B6 <i>H-2^{bf}</i> <i>bf bbb b b</i>	Immune	32.1	28.4	54.6	26.4	54.4	28.9	51.7	25.7	59.1	20.1
	Normal	24.1	—	29.4	25.2	28.0	28.2	20.2	22.4	20.0	16.8
B10 <i>b bbb b b</i>	Immune	59.6	26.2	59.5	24.5	52.4	29.2	58.9	26.7	88.5	19.7
	Normal	24.1	—	29.4	25.2	28.0	28.2	20.2	22.4	20.0	16.8
B10.A(5r) <i>b bbd d d</i>	Immune	60.2	27.6	22.7	23.6	27.1	29.6	32.9	25.0	62.7	18.9
	Normal	24.2	—	24.1	21.8	22.7	24.3	27.7	24.3	25.2	17.1
B10.A(2r) <i>k kkd d b</i>	Immune	26.2	25.5	78.7	22.9	60.7	26.7	44.8	22.6	73.2	20.1
	Normal	24.1	—	29.4	25.2	28.0	28.2	20.2	22.4	20.0	16.8

* 2×10^6 spleen cells were assayed on 1×10^5 target cells in each well for 5 h at 37°C.

† Mice were injected i.p. with 2×10^7 PFU of vaccinia virus 6 days previously.

§ Means of triplicates; the SEM were between 1.2 and 3.1.

|| Significantly ($P < 0.05$) different from immune spleen cells on infected and normal spleen cells on infected or normal target cells (where applicable).

H-2^{bf} immune cells never caused statistically significant lysis of B10.A(5r) targets, a small degree of cross-reactivity with *H-2K^b* as indicated by the repeatedly greater lysis as compared with *H-2^{ba}* could not be completely excluded.

The lack of significant mutual lytic interactions of B10.A(5r) and B6 *H-2^{ba}* or B6 *H-2^{bf}* could be from either deletion or modification of the cell surface structure involved. It was therefore important to demonstrate positively the existence of two distinct specific cytotoxic activities, one associated with *H-2^{ba}* or *H-2^{bf}* and one with *H-2D^b* by using cold target competition experiments (16). In homozygous mice, two sets of virus-specific cytotoxic T cells are generated, associated with structures coded in the *K* or *D* region of the *H-2* gene complex. ⁵¹Cr release caused by either set can be decreased individually by adding unlabeled *H-2K* or *H-2D* compatible infected target cells. An excess of approximately nine unlabeled competitors per labeled target cell has been shown to block reliably, even if effector cells were in excess (16). For technical reasons this experiment was only performed with vaccinia virus.

Addition of unlabeled infected B10.A(2r) macrophages did not inhibit ⁵¹Cr release by immune B6 *H-2^{ba}* cells from *H-2^{ba}* targets but did decrease release by immune B10 or B6 *H-2^{bf}*, as compared with added normal macrophages; the same infected competitors did not significantly interfere with the ⁵¹Cr release caused by B10 immune cells on B10 targets (Table III). Comparable results were obtained on *H-2^{bf}* targets.

These results clearly show the presence of two sets of cytotoxic T cells in the *H-2* mutant mice, one being associated with *H-2K^{ba}* or *H-2K^{bf}*, the other with *H-2D*. The results obtained also indicate that vaccinia-immune T cells cytolytic for virus infected *H-2K^{ba}* or *H-2K^{bf}* target cells do not cross-react to a great degree. The activity of *H-2K^{ba}* immune cells on *H-2K^{bf}* targets was virtually abrogated

TABLE III
*Demonstration of Cytotoxic T-Cell Activity against Virus-Specific Altered H-2K^{ba} and H-2K^{bf} by Cold Target Competitive Inhibition Assays**

Donors of vaccinia-immune spleen cells†	⁵¹ Cr-labeled target vaccinia infected	% ⁵¹ Cr release from macrophage-target cold competitors [B10.A(2r)]§		
		None	Normal	Infected
B6 H-2 ^{ba}	B6 H-2 ^{ba}	94.1	82.7	79.2
B6 H-2 ^{bf}	B6 H-2 ^{ba}	59.6	56.5	45.4
B10	B6 H-2 ^{ba}	62.7	63.6	42.9
Medium	B6 H-2 ^{ba}	34.4		
B6 H-2 ^{ba}	B6 H-2 ^{bf}	68.1	66.2	47.0
B6 H-2 ^{bf}	B6 H-2 ^{bf}	74.5	70.2	65.2
B10	B6 H-2 ^{bf}	89.2	84.5	59.3
Medium	B6 H-2 ^{bf}	45.1		
B10	B10	94.9	87.4	85.3
B10.A(5r)	B10	85.1	81.0	79.3
Medium	B10	39.2		

* Activity against other targets and control values of this experiment are reported in Table II. 2×10^6 spleen cells were assayed on 3×10^4 target cells for 5 h at 37°C.

† From mice injected i.p. 6 days previously with 2×10^7 PFU of vaccinia.

§ Unlabeled normal or infected competitor macrophages were added eight times in excess of the labeled target macrophages. Results are expressed as means of triplicates; the SEM varied from 2.3 to 4.7.

|| Significantly smaller than with uninfected competitors.

by the addition of cold B10.A(2r) competitors, indicating that the measurable activity in this combination is due only to activity associated with H-2D.

Discussion

Immune spleen cells from virus-infected H-2 mutant mice B6 H-2^{ba} and B6 H-2^{bf} cannot lyse infected B10.A(5r) macrophage targets expressing the wild-type H-2K^b and the incompatible H-2D^d and vice versa. The mutation is not a deletion, as specific cytotoxic activity solely associated with H-2K^{ba} or H-2K^{bf} could be demonstrated.

The results demonstrate that the cell surface structure, which is central in the apparent restriction of the lytic activity of virus-specific cytotoxic T cells is coded at a single locus within the K and by analogy probably also in the D region of the H-2 gene complex. The locus within the K region of the H-2 gene complex is defined by the two kinds of H-2 mutant mice tested, which both differ at the same single cistron from wild-type B6 mice. The data also confirm for vaccinia virus that compatibility at the I region is neither required nor alone sufficient for virus-specific cytolytic interactions.

How can these findings help to distinguish between two, probably mutually exclusive, models used to explain the requirement for compatibility at H-2K or H-2D for virus-specific and other T-cell-mediated cytolytic interactions (1-7, 16, 17)? First, the "altered self" concept proposes that T cells are sensitized not against viral antigens alone, but against structures coded in the K or D region of H-2 which are modified by virus (1-3) [or by TNP (4, 5) or minor histocompatibility antigens (6)]. Second, T cells may be specific for viral antigens, but lytic activity requires a second "physiological interaction" between like H-2 antigens on target and killer cells (1-7, 17).

Of the two, the altered self concept most readily accommodates experimental findings obtained with cytotoxic T cells from virus infections, (1–3, 16) or generated in vitro against TNP-modified syngeneic spleen cells (4–6) as well as reactivity against alloantigen. However, no direct proof, like biochemical isolation of altered self entities, is available as yet.

The results with the *H-2* mutant mice correlate readily with the idea that cytotoxic T cells are specific for altered self structures coded in the *K* or *D* regions. The *H-2* mutants would thus genetically map the modifiable self marker located in the *K* region.

Nevertheless, the physiological interaction model cannot be excluded. It can be argued that besides being simultaneously subject to allelic exclusion, to mutual *K*- and *D*-region exclusion of physiological interaction structures (coded within the *K* region for like *H-2K* and *D* region for like *H-2D*), and to clonal expression, the physiological recognition unit and the self marker are part of the same structure. However, this seems unlikely, since structures coded in the *K* and *D* regions relevant to virus-specific and allogeneic T-cell interactions are codominantly expressed on the cell surface (2, 10, 16). Within the physiological interaction model, another design that accommodates the results would be to postulate that relevant self structures can mutate only within limits allowed by probably evolutionarily ancient genes common to all mice, which code for mutual interaction structures; these genes would either code for a great variety of such structures or could be subject to a high rate of somatic mutation (18). Such an argument would circumvent the difficult postulate of parallel mutation of self marker and interaction structures (19) and could mean that the interaction structure is coded elsewhere than in the *K* or *D* regions of *H-2*. After a permissive mutation of the self marker evolves, interaction with the wild-type self structure would be impossible since a different one of possible interaction structures would be phenotypically expressed.

Although the altered self concept offers a simpler and more generally applicable explanation of the available experimental data, all evidence is circumstantial. The question, which of the two models is correct, is thus still open.

Summary

Lymphocytic choriomeningitis or vaccinia virus-immune spleen cells of *H-2* mutant mice carrying a point mutation in the *K* region (B6 *H-2^{ba}*, B6 *H-2^{bf}*) cannot lyse infected wild-type *H-2K^b* targets and vice versa. Yet, cytotoxic T cells specific for infected *H-2K^{ba}* or *H-2K^{bf}* targets are generated during virus infections as shown by cold target competition experiments. The critical structure for the apparent restriction by the *K* or *D* regions of the *H-2* gene complex of cytolytic interactions between T cells and virus-infected target cells are therefore each coded, at least as shown for the *K* region, by a single cistron. This finding is most readily accommodated within the altered self concept (postulating that T cells are specific for virus-modified self structures) but cannot exclude the possibility of a physiological interaction mechanism being responsible for the apparent *H-2* restriction of virus-specific cytotoxic T cells.

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