Brief Definitive Report

H-2 COMPATIBILITY IS REQUIRED FOR T-CELL-MEDIATED LYSIS OF TARGET CELLS INFECTED WITH LYMPHOCYTIC CHORIOMENINGITIS VIRUS

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Adult CBA/H mice injected with lymphocytic choriomeningitis (LCM) virus generate specifically sensitized thymus-derived (T) lymphocytes which are cytotoxic, in an in vitro ^{51}Cr-release assay, for C3H mouse L929 fibroblasts (L cells) expressing LCM viral antigen (1, 2). When a variety of inbred mouse strains were immunized it was found that only those sharing at least one *H-2*^{k} haplotype with the target L cells caused high levels of ^{51}Cr release (3, 4). Furthermore, specific lysis of virus infected macrophages (3) from *H-2*^{k} or *H-2*^{d} mice was recognized only in the syngeneic combinations. This led to the interpretation that cytotoxic T-cell activity in LCM is, as is the T-cell helper effect (5, 6), restricted by the *H-2*-gene complex. Subsequent experiments have shown that the same constraint applies to T-cell-mediated lysis of targets infected with ectromelia virus,[1] and to in vivo T-cell effector activity in LCM, listeriosis, and ectromelia (7–9). Also, lysis of trinitrophenyl-modified lymphocytes by sensitized T cells has been found to be similarly restricted (10).

The present paper rigorously establishes that identity at the *H-2*-gene complex is essential for maximal T-cell-mediated lysis of LCM virus-infected targets. Evidence is also presented which indicates that compatibility at either *H-2K* or *H-2D* is sufficient.

Materials and Methods

Mouse Strains. CBA/H, C57BL, BALB/c, and A/J mice were from our own colonies. The B10.A, A.BY, CSW, and C3H/DiSn strains were kindly supplied by Dr. H. O. McDevitt of Stanford University, Stanford, Calif.

Immunization. Adult mice (8–12 wk) were injected either intracerebrally (i.c.) or intravenously (i.v.) with 300 LD_{50} or 2,000 LD_{50} of WE3 LCM virus, respectively (1). Except where otherwise stated, immune spleen cells were prepared (1) from clinically affected mice dosed i.c. 7 days previously. Those inoculated i.v. were killed at intervals from 5 to 13 days.

Target Cells. Mouse embryo fibroblasts and L cells were grown in Eagle's minimal essential medium (F15, Grand Island Biological Co., Grand Island, N. Y.) plus 10% fetal calf serum (FCS). DBA/2 P815 mastocytoma cells were maintained in Dulbecco's modified Eagle's medium (H16, GIBCO) plus 10% FCS. Fibroblasts were dispensed into plastic tissue culture trays and infected with a high multiplicity of WE3 LCM virus at 24 h before labeling with ^{51}Cr (3). The mastocytoma cells were cocultivated, for 24 h, with WE3-infected L-cell monolayers, and have since been maintained as an infected line.

[1] Gardner, I., N. A. Bowern, and R. V. Blanden. Cell-mediated cytotoxicity against ectromelia virus-infected target cells III. Role of the *H-2*-gene complex. Manuscript submitted for publication.

Cytotoxic Assay. The ^{51}Cr-release assay used has been described (2). Briefly, virus-infected or normal target cells were labeled with ^{51}Cr and overlayed with immune or normal spleen cells at a ratio of 30:1. Assays were incubated for 16 h at 37°C, the supernates pipetted, and residual cells lysed with water. Results are expressed either as mean ± SEM percent ^{51}Cr release for four replicates (2), or as numbers of target cells specifically lysed (11).

Results

Dose Response. Virus infected and normal L cells (*H-2^k*) and mastocytoma cells (*H-2^d*) were grown in suspension cultures, labeled with ^{51}Cr, and mixed with varying concentrations of syngeneic or allogeneic immune or normal spleen cells. Reciprocal requirement for *H-2* compatibility was demonstrated at all ratios (Fig. 1). Significant ^{51}Cr release was observed in the syngeneic systems at levels as low as one immune spleen cell to one virus-infected target, but was not found at 100:1 in allogeneic combinations.

Fibroblast Targets. Spleen populations from *H-2^k*, *H-2^d*, and *H-2^b* mice were assayed on syngeneic or allogeneic fibroblast monolayers. Maximal lysis was, in every case, recognized when virus infected targets were exposed to syngeneic immune spleen cells (Table I). However, there was considerable nonspecific ^{51}Cr release from the 3T3 fibroblasts, and background cytotoxicity varied greatly. Various explanations for this are that it is mediated by cells other than T cells, that there is degeneracy of the T-cell response,[1] or that virus in the immune spleen population is infecting normal targets.

Sufficiency of H-2K or H-2D Compatibility. Virus-infected and normal *H-2^k*

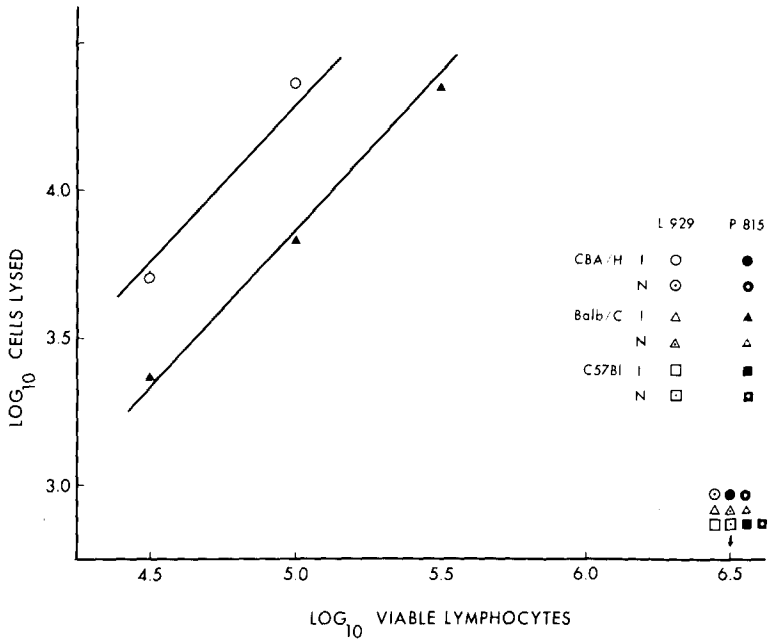

FIG. 1. Numbers of targets lysed (11) by exposure to increasing numbers of immune (I) or normal (N), syngeneic or allogeneic, spleen cells. Only the linear portions are shown, the plateau occurring at higher concentrations of immune cells having been described elsewhere (2).

TABLE I

Percent ^{51}Cr Release from Different Fibroblast Monolayers by Immune or Normal Spleen Cells

Mouse strain	Spleen cells	L cells (*H-2*^{k})		DBA/2 3T3 (*H-2*^{d})		C57BL 3T3 (*H-2*^{b})	
		Infected	Normal	Infected	Normal	Infected	Normal
CBA/H (*H-2*^{k})	Immune	86.7 ± 3.1	26.9 ± 1.0	26.3 ± 1.8	26.3 ± 2.9	40.0 ± 2.0	42.4 ± 3.0
	Normal	27.3 ± 0.7	21.7 ± 0.7	19.6 ± 0.3	25.0 ± 1.5	34.8 ± 0.3	38.1 ± 2.0
BALB/c (*H-2*^{d})	Immune	27.9 ± 1.1	20.7 ± 1.6	53.0 ± 4.7	22.5 ± 3.8	33.8 ± 3.2	22.8 ± 0.4
	Normal	20.5 ± 1.6	27.9 ± 0.6	19.1 ± 0.6	23.4 ± 0.8	25.6 ± 3.4	44.6 ± 0.6
C57BL (*H-2*^{b})	Immune	34.9 ± 1.4	35.1 ± 2.7	46.1 ± 3.8	38.1 ± 1.8	63.0 ± 4.0	34.2 ± 3.4
	Normal	25.3 ± 6.1	23.1 ± 2.4	19.1 ± 1.3	19.7 ± 1.4	29.0 ± 4.0	28.6 ± 6.0

(L cell) and *H-2*^{d} (P815 and DBA/2 fibroblast) targets were exposed to spleen cells from CBA/H (*H-2*^{k}), A/J (*H-2*^{a}), or BALB/c (*H-2*^{d}) mice. *H-2*^{a} is a recombinant between *H-2*^{k} and *H-2*^{d}, having K-end specificities of *H-2*^{k} and D-end specificities of *H-2*^{d} (12). Maximal specific ^{51}Cr release was found when immune T cells and virus-infected targets were compatible for either *H-2K* or *H-2D* (Fig. 2). Identity at both loci is apparently not essential.

Congenic Strains. Due to the generosity of Dr. H. O. McDevitt we were able to examine mice which differed only at the *H-2*-gene complex (Table II). In every case high levels of specific lysis occurred only in *H-2K* or *H-2D* compatible systems. Differences elsewhere in the genotype were irrelevant.

Discussion

In our initial discussion of this phenomenon we considered that requirement for *H-2* compatibility may reflect one of two mechanisms (3, 4). Either the *H-2*-gene complex is associated with some physiologic self-recognition system, or T cells may be sensitized to "altered self" components. Allogeneic inhibition (13) has been excluded in both in vitro and in vivo studies using $F_1 \rightleftarrows$ parent combinations (3, 7).

Subsequent experiments have shown that there are T cells of at least two specificities in LCM-immune F_1 mice, each associated with one parent *H-2* type (14). We have thus argued (14) that the physiologic interaction model can only be supported if there is allelic exclusion of the T-cell recognition system. A more likely explanation would seem to be that the T cell is sensitized to altered self antigens, or to some complex of virus plus self components. Recognition of chemically (10) or virally modified cell surface structures, or of alloantigens (11), can thus be explained by the same altered self concept (14).

Whichever is ultimately shown to be correct, our results indicate that compatibility at either the K or the D end of the *H-2*-gene complex is sufficient. In this respect LCM apparently differs from the T-cell helper system (6), where cooperation has been related to mutuality at the K end alone. Alloantigens associated with *H-2K* and *H-2D* cap independently (15). The finding for the LCM model can thus readily be accommodated within the virus plus self, or

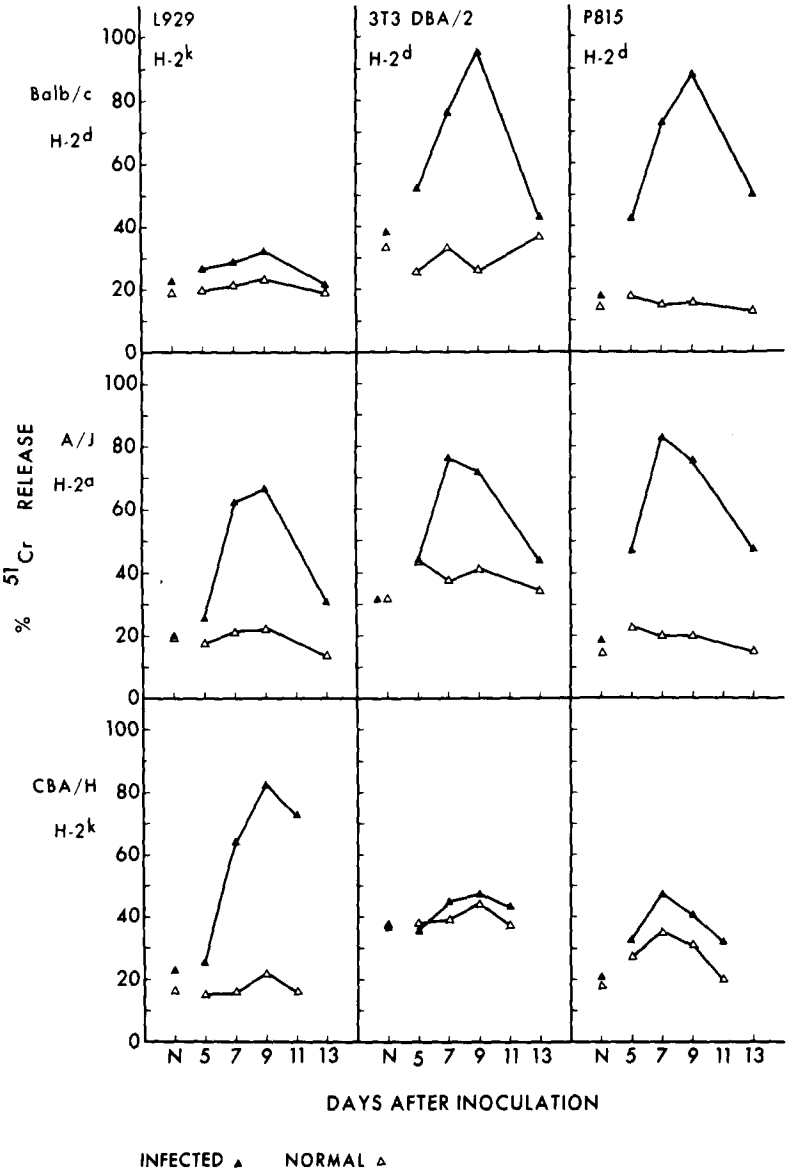

FIG. 2. Lysis of virus-infected or normal target cells of *H-2*^{k} or *H-2*^{d} antigenic specificities by immune or normal spleen cells from BALB/c (K^d-D^d), A/J (K^k-D^d) or CBA/H (K^k-D^k) mice.

altered self, concept, "self" being K- or D-end antigens. Examination of the mouse strains investigated so far (3) indicates that the self components involved may be the serologically-defined *H-2* private specificities (12), or structures coded for by genes linked closely to them.

Summary

Maximal cell-mediated lysis of targets infected with lymphocytic choriomeningi-

TABLE II

Evidence that Maximal ^{51}Cr Release Requires H-2K or H-2D Compatibility between Immune Spleen Cells and Virus-Infected Target Cells

Mouse strain	*H-2*		Back-ground strain	L cells (*H-2^k*)		P815 (*H-2^d*)	
	K	D		Infected	Normal	Infected	Normal
A/J	k	d	A/J	51.1 ± 1.4	22.1 ± 0.7	74.5 ± 1.3	28.3 ± 0.9
A.BY	b	b	A/J	28.0 ± 1.3	21.8 ± 0.9	37.5 ± 1.9	36.1 ± 1.3
B10.A	k	d	C57BL	64.5 ± 2.3	21.7 ± 2.3	82.5 ± 3.1	27.3 ± 0.9
C57BL	b	b	C57BL	37.1 ± 3.4	30.4 ± 1.4	30.4 ± 2.6	33.1 ± 1.2
CSW	b	b	C3H	31.3 ± 2.0	22.3 ± 0.7	39.1 ± 0.9	39.0 ± 1.7
C3H/DiSn	k	k	C3H	48.8 ± 2.6	18.1 ± 0.7	NT	NT

tis virus occurs only within a *H-2* compatible system. Syngeneic immune spleen cells are at least 100 times as effective as are allogeneic lymphocytes. Reciprocal restriction of cytotoxic T-cell activity has been shown to operate between *H-2^k*, *H-2^d*, and *H-2^b*. Experiments with congenic mice have localized the effect to the *H-2*-gene complex. Furthermore, the observation that lymphocytes from *H-2^a* mice cause high specific ^{51}Cr release from either *H-2^k* or *H-2^d* virus-infected cells, indicates that identity at either the K or the D end of the *H-2*-gene complex is sufficient for this lytic interaction.

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