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

Evaluation of the Role of *H-2I* Region and Non-*H-2* Genes in Regulating Immune Response*

By ROLF M. ZINKERNAGEL, MALCOLM B. C. DUNLOP, ROBERT V. BLANDEN, PETER C. DOHERTY, AND DONALD C. SHREFFLER

(From the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037; the Department of Microbiology, The John Curtin School of Medical Research, Australian National University, Canberra, Australia; The Wistar Institute, Philadelphia, Pennsylvania 19104; and the Department of Genetics, Washington University, St. Louis, Missouri 63110)

Acute murine lymphocytic choriomeningitis (LCM)[1] is a well documented example of predominantly cell-mediated immunopathology. The experimental evidence leading to this conclusion has been reviewed in extenso recently (1–3). The pathological process in vivo can be monitored in vitro by measuring LCM virus (LCMV)-specific cytotoxic activity of thymus-derived lymphocytes (T cells) for LCMV-infected target cells (4–7). That these cytotoxic T cells may be directly involved in destroying the meninges and ependymal cells is suggested by the fact that they can be isolated with great specific reactivity from cerebrospinal fluid of clinically affected mice (8).

Oldstone et al. (9) reported that a small but statistically significant susceptibility difference of two mouse strains could be linked to their *H-2* type, possibly reflecting *Ir* gene control. More recently, with the mouse strains available now, this difference no longer appears to be statistically significant (10). Nevertheless, it was during the attempt to correlate increased susceptibility to acute LCM with the capacity of various mouse strains to generate LCMV-specific T cells with greater cytotoxic activity and quicker than less susceptible mice that the unexpected *H-2* restriction of cytolytic activity was originally found (2, 11).

Many other T-cell functions were known or were subsequently shown to be restricted by the *H-2* gene complex in a similar or different way. For the T-helper function, T cells and bone marrow-derived lymphocytes (B cells) clearly require compatibility at the *I* but not at the *K* or *D* region of *H-2* (12, 13).

* This is publication no. 1055 from the Department of Immunopathology, Scripps Clinic and Research Foundation. Part of this research was supported by U. S. Public Health Service grants AI-07007 and AI-12734.

[1] *Abbreviations used in this paper:* J774, BALB/c fibrosarcoma cells; L cells, L929 C3H fibroblasts; LCM, lymphocytic choriomeningitis; LCMV, lymphocytic choriomeningitis virus; LD_{50}, lethal dose for 50% of recipients; LU, lytic unit; P815, DBA/2 mastocytoma cells; PFU, plaque-forming units.

Exceptions to this rule were subsequently found by experiments using alloantigen-tolerant T cells (14, 15). Also, effector T cells involved in delayed type hypersensitivity to fowl γ-globulin must be *I*-region compatible for optimal expression of function (16). Quite different, LCMV, pox-, or Sendai virus-specific cytolytic interactions require *K*- or *D*-region compatibility (17–19). In an earlier report it was shown that *I*-region compatibility alone was not or only questionably sufficient for lysis to occur (17). However, it is not known whether the target cells used in these studies express any cell surface structure coded by the *H-2I* region, such as Ia antigens.

The role of structures coded by the *I*-region genes for expression of cytolytic effector function was therefore reassessed using macrophage target cells, which are known to possess *Ia* cell surface specificities (20–22). Furthermore, the regulating role of *I*-region genes on the capacity to generate virus-specific cytotoxic T cells in vivo was investigated. No obvious role for a structural or regulatory *I*-region gene product could be detected. However, the results indicate that non-*H-2* genes may greatly influence immune responsiveness measured by virus-specific cytotoxicity.

Materials and Methods

Mice. 6- to 9-wk-old CBA/H, C57BL/6, BALB/c, BALB/c × C57BL/6 F_1, and DBA/2 were either bred in the colonies maintained at the Australian National University (Tables II–IV) or were purchased from The Jackson Laboratory, Bar Harbor, Maine (Tables V–VII). SJL/J, DBA/1, B10.D2, B10.A, B10.A(2r), B10.A(4r), B10.A(5r), and C57BL/10 were from The Jackson Laboratory. A.TFR3, A.TFR5, A.TL, A.TH, C3H.OH, C3H.OL, D2.GD, and DA/HuSn were from colonies originating from the Department of Human Genetics, University of Michigan Medical School. The *H-2* haplotypes of the mice used are summarized in Table I (22).

Virus and Immunization. Both the immunization procedures (6, 7, 17, 19) and the strains of LCMV (6, 7, 17) of ectromelia virus (17) and the vaccinia virus used (18) have been described in detail elsewhere. Mice were, unless otherwise stated, injected intravenously (i.v.) with 10^3 intracerebral (i.c.) LD_{50} (lethal dose for 50% of recipients) of the WE strain of LCMV or 2–5 × 10^4 plaque forming units (PFU) of a virulent Hampsted egg ectromelia virus or 2 × 10^7 PFU of WR vaccinia virus. The vaccinia virus was a gift of Dr. W. K. Joklik, Duke University, Durham, N. C. LCMV immune spleen cells were usually harvested at 7 days, pox virus immune spleen cells at 6 days after infection.

^{51}Cr Release Assay. The techniques employed and the cell lines used as targets have been described (4–6, 7, 19, 24–28). Peritoneal macrophages either from unstimulated mice (11) or from mice injected 4 days previously with thioglycollate were plated into flat bottom 96-well hemagglutination trays (Falcon Plastics, Oxnard, Calif.) at 2 × 10^5 cells/well. The following day monolayers were infected with LCMV for 24 h or with pox viruses for 6 h and labeled with ^{51}Cr. Immune or normal spleen cells were overlaid usually at a ratio of 30 to 1 target cell and incubated at 37°C. ^{51}Cr release was determined 5–14 h later. Release by water represents 100% release; results are not corrected for spontaneous ^{51}Cr release. Means ± SEM of triplicates were determined and compared by using Student's *t* test.

Results

Genetic Mapping with Macrophage Targets. Reciprocal assays were done using immune spleen cells and virus-infected macrophages from *H-2* recombinant and original type mouse strains (Tables I and II). Significant lysis by virus immune spleen cells on infected targets, as compared with immune cells on uninfected or normal cells on infected targets, was recognized when donors of

TABLE I
*H-2 Haplotypes of the Mice Used**

	K	I			*S*	*D*
		A	*B*	*C*		
BALB/c, B10.D2	*d*	*d*	*d*	*d*	*d*	*d*
CBA/H	*k*	*k*	*k*	*k*	*k*	*k*
SJL/J	*s*	*s*	*s*	*s*	*s*	*s*
C57BL/6J, C57BL/10	*b*	*b*	*b*	*b*	*b*	*b*
DBA/1J	*q*	*q*	*q*	*q*	*q*	*q*
A.TFR3	*f*	*f*	*f*	*f*	*s*	*d*
A.TFR5	*f*	*f*	*f*	*f*	*k*	*d*
A.TL	*s*	*k*	*k*	*k*	*k*	*d*
A.TH	*s*	*s*	*s*	*s*	*s*	*d*
B10.A	*k*	*k*	*k*	*d*	*d*	*d*
B10.A(2r)	*k*	*k*	*k*	*d*	*d*	*b*
B10.A(4r)	*k*	*k*	*b*	*b*	*b*	*b*
B10.A(5r)	*b*	*b*	*b*	*d*	*d*	*d*
C3H.OH	*d*	*d*	*d*	*d*	*d*	*k*
C3H.OL	*d*	*d*	*d*	*d*	*k*	*k*
D2.GD	*d*	*d*	*b*	*b*	*b*	*b*
DA/HuSn	*q*	*q*	*q*	*q*	*q*	*s*

* From Shreffler and David (23).

virus immune spleen cells and infected macrophage targets were compatible at the *K* or *D* regions of the *H-2* gene complex. *K*-region compatibility alone, as for A.TL on SJL, was sufficient, as was *D*-region homology alone. (A.TL → BALB/c, CBA/H → C3H.OH, BALB/c → A.TH, Tables II and VIII). Cytolysis was not observed when donors of T cells and targets shared identity only in the *I* region, as for A.TL → CBA/H, and BALB/c → B10.A(2r) which are compatible at the *I-C* and *S* regions.

Effect of Various H-2I-Region Specificities on the Generation and Expression of Virus-Specific Cytotoxicity Associated with H-2D^d. The possible role for *I*-region genes in the generation in vivo of virus-specific cytotoxic T cells was investigated using *H-2* recombinant mice. Several *H-2* recombinant mouse strains derived from the A strain share the *D*^{d} allele, but possess different *I* and *K* regions of other than *H-2*^{d} origin. The virus-specific cytotoxic activity generated in vivo was assessed in vitro by determining lytic units (LU), i.e., the average number of spleen cells necessary to lyse 1/3 of a standard number of target cells (23). The smaller the value, the relatively more active is the tested spleen cell population.

The LU generated during LCMV infection in the various mouse strains differed maximally by a factor of 1.7, while the comparable value for ectromelia was 2.5 (Table III). These variations do not seem to reflect specific non-*H-2* differences as mice of both the A strain and B10 non-H-2 background show the spectrum of activities with both virus infections.

Relative cytotoxic activity of BALB/c immune spleen cells on *H-2*^{d} virus-infected target cells tended to be low, even though the whole of the *H-2* gene

TABLE II

I-Region Compatibility is Neither Sufficient Nor Necessary for Virus-Specific Cytolysis of Macrophage Targets

Immunogen*	Mouse strain	Target infection	Percent ^{51}Cr release from macrophages‡				
			SJL/J	CBA/H	BALB/c		
Ectromelia virus	A.TL	Ectromelia	53.1 ± 3.1§	23.5 ± 0.7	62.7 ± 2.2‖		
	CBA/H	Ectromelia	18.5 ± 0.8	57.8 ± 3.6‖	32.8 ± 3.3‖		
	A.TL	Nil	25.2 ± 1.1	24.7 ± 0.4	21.9 ± 1.3		
	CBA/H	Nil	25.4 ± 2.3	20.3 ± 0.6	19.9 ± 2.5		
LCMV	A.TL	LCMV	45.1 ± 1.8‖	28.7 ± 1.0	68.3 ± 1.3‖		
	CBA/H	LCMV	29.8 ± 1.2	62.6 ± 3.0‖	26.5 ± 0.8		
	A/J	LCMV	28.6 ± 0.8	51.7 ± 1.3‖	68.6 ± 1.8‖		
	Normal CBA/H	LCMV	28.5 ± 0.2	23.3 ± 0.4	30.5 ± 1.4		
			C3H.OH	B10.A(2r)	DA/HuSn	A.TH	A.TL
Ectromelia virus	BALB/c	Ectromelia¶	52.2 ± 2.1‖	46.6 ± 3.1	44.7 ± 4.2	46.9 ± 2.0‖	NT
	CBA/H	Ectromelia	45.3 ± 3.4‖	72.1 ± 4.7‖	40.3 ± 0.8	35.0 ± 1.3	NT
	SJL/J	Ectromelia	40.4 ± 2.8	51.2 ± 3.2	65.3 ± 1.8‖	63.0 ± 2.5‖	NT
	C57BL/6J	Ectromelia	38.9 ± 2.5	67.9 ± 2.2‖	46.8 ± 1.7	37.4 ± 1.1	NT
	DBA/1	Ectromelia	38.7 ± 2.5	47.2 ± 4.0	70.1 ± 4.1‖	41.5 ± 4.5	NT
	Nil	Ectromelia	33.4 ± 1.0	51.1 ± 4.2	39.7 ± 1.5	35.3 ± 1.3	NT
LCMV	BALB/c	LCMV	82.8 ± 4.8‖	52.3 ± 5.0	40.1 ± 7.1	90.4 ± 5.4‖	87.6 ± 8.0‖
	CBA/H	LCMV	96.4 ± 7.7‖	78.4 ± 3.2‖	42.9 ± 8.4	60.1 ± 5.1	58.8 ± 1.5
	C57BL/6	LCMV	NT**	70.3 ± 4.7‖	42.7 ± 9.6	66.7 ± 5.8	NT
	DBA/1	LCMV	NT	NT	50.6 ± 3.6‖	57.8 ± 6.3	NT
	SJL/J	LCMV	57.1 ± 5.1	54.9 ± 8.4	60.0 ± 5.9‖	83.8 ± 6.1‖	68.2 ± 2.5‖
	Normal C57BL/6	LCMV	50.9 ± 10.1	48.7 ± 3.8	40.5 ± 2.5	66.6 ± 7.0	55.4 ± 4.8

* Mice were infected i.v. with 2×10^4 PFU attenuated ectromelia virus or 2×10^3 i.c. LD_{50} of WE3 LCMV, and spleen cells were harvested respectively 6 or 7 days later.

‡ Immune or normal spleen cells were assayed at a spleen cell to target ratio of 30:1 for 10 h at 37°C.

§ Means ± SEM of triplicates. Release by immune spleen cells on infected targets was recorded as significant only when statistically greater than release caused on uninfected targets or of normal spleen cells on normal or infected targets.

‖ Significantly different from controls ($P < 0.05$).

¶ ^{51}Cr release of immune spleen cells on uninfected target cells varied from 29.7 to 36.5 on C3H.OH, from 30.1 to 46.7 on A.TH, from 27.7 to 35.6 on B10.A(2r), and from 32.9 to 41.4 on DA/HuSn targets.

** NT, not tested.

complex is shared. Thus, there was no indication that *I*-region compatibility conferred enhanced cytolytic capacity, especially as, in this case, donors of T cells and target cells are also compatible at *H-2K*^{d}. When the total cytotoxic activity per spleen was calculated for LCMV-infected mice, differences up to 6.5-fold were noticed between strains, e.g., A.TL and B10.A. Most of these differences are, however, explained mainly by the differences in mean total spleen cell numbers.

Low Responsiveness of D2.GD Mice to LCMV. When a number of *H-2* recombinant mouse strains were assayed for LCMV-specific cytotoxicity, D2.GD (*H-2K*^{d}*D*^{b}) mice were found to have little, if any, activity against LCMV-infected *H-2*^{d} targets. Further experiments revealed that D2.GD 7-day immune spleen cells were not active at 30:1 against *H-2K*^{d} compatible targets (C3H.OL or BALB/c macrophages), but were lytic for *H-2D*^{b} compatible targets (Table IV). Although it appeared from these results that D2.GD cells might not efficiently express antigen-associated with *H-2K*^{d}, it became obvious, in a second experiment, that they were comparable to other *K*^{d} targets (Table V).

TABLE III

Cytotoxic Activity of Virus Immune T Cells of Various I-Region Specificities for H-2D^d Compatible Virus-Infected Mastocytoma P815 (H-2^d) Target Cells

Mouse strain	*H-2* haplotype	LCMV*				Ectromelia* LU (10^5)
		Exp. 1			Exp. 2	
		LU‡ (10^5)	Spleen cells per spleen (10^7)	LU/ spleen (10^2)	LU (10^5)	
A.TFR3	*f fff s d*	1.4	3.0	2.2	2.7	1.7
A.TFR5	*f fff k d*	1.5	9.0	6.0	2.5	NT
A.TH	*s sss s d*	2.2	7.0	3.2	3.5	3.4
A.TL	*s kkk k d*	2.4	2.2	0.7	4.0	4.0
B10.A	*k kkd d d*	1.5	9.0	6.0	2.5	3.1
B10.A(5r)	*b bbd d d*	2.2	8.5	3.9	3.5	1.6
BALB/c	*d dddd d*	2.1	12.0	5.7	NT	NT

* Spleen cells from mice infected for 7 days with 2×10^3 i.c. LD_{50} of WE3 LCMV or for 6 days with 2×10^4 PFU ectromelia virus were assayed at various ratios (30:1–1:1) on virus-infected P815 target cells.

‡ Mean number of immune spleen cells needed to specifically release 33% of the ^{51}Cr incorporated by 5×10^4 target cells (24).

TABLE IV

Differential Capacity of H-2K^d Compatible D2.GD and C3H.OL Mice to Generate Cytotoxic T Cells in LCM Infection

Spleen cells		Percent ^{51}Cr release from LCMV-infected macrophages*				
		D2.GD	C3H.OL	BALB/c	C57BL	CBA/H
Immune‡	D2.GD	43.6 ± 1.4§	28.3 ± 2.1	29.2 ± 1.1‖	43.5 ± 1.8‡	22.3 ± 0.6
Normal	D2.GD	23.5 ± 0.8	24.9 ± 1.6	26.1 ± 1.5	25.0 ± 1.7	22.9 ± 0.6
Immune	C3H.OL	26.2 ± 1.5	43.5 ± 2.3‡	38.8 ± 2.4‖§	NT	30.5 ± 0.4§
Normal	C3H.OL	24.1 ± 0.4	24.7 ± 1.8	26.8 ± 0.9	NT	19.9 ± 0.7
Immune	BALB/c	38.7 ± 0.5§	NT	61.7 ± 2.4§	31.7 ± 1.2	NT
Normal	BALB/c	34.2 ± 0.5	NT	27.3 ± 2.0	33.2 ± 1.1	NT
Immune	BALB/c × C57BL F_1	60.0 ± 1.7§	36.1 ± 2.5	74.8 ± 1.1§	70.2 ± 1.1§	28.2 ± 1.0

* Spleen cell to target ratio 30:1, incubated for 7 h at 37°C.

‡ Mice were infected with 2×10^3 i.c. LD_{50} of WE3 LCMV 7 days previously.

‖ When immune spleen cells from four individual D2.GD immune mice were assayed on LCMV-infected DBA/2 (P815) mastocytoma cells, levels of ^{51}Cr release ranged from 21.8 ± 0.8 to 23.5 ± 0.8 compared with 35.0 ± 1.6 to 39.3 ± 1.3 for C3H.OL immune T cells. Values for normal spleen cells were 20.4 ± 0.7 and 18.2 ± 0.9, respectively.

§ Significantly different from immune spleen cells on uninfected targets and/or normal spleen cells on infected targets.

This low responsiveness under the conditions tested was specific for LCMV, as pox virus infection provoked high cytotoxic activity (Table V).

D2.GD mice are derived from the DBA/2 strain which, when assayed for LCMV-specific cytotoxicity, were also found to be low or nonresponders. Individual mice were infected with a standard dose of 10^3 LD_{50} i.c. and assayed 7 or 9 days later. During this time interval maximal cytotoxic activity was usually observed with all strains tested so far (17, 24) (Table VI). Immune spleen cells from DBA/2 and D2.GD mice caused virtually no lysis of K^d targets, but D2.GD cells were lytic for D^b target cells although much less lytic than were those from C57BL/6.

TABLE V

Comparison of the Cytotoxic Activity Generated in Various Mouse Strains Infected with LCMV or Vaccinia Virus

Spleen cells		Percent ^{51}Cr release from LCMV-infected macrophages*			
		D2.GD	C3H.OH	BALB/c	C57BL
LCMV-immune‡	D2.GD	NT	37.2 ± 1.4§‖	31.7 ± 1.6	45.3 ± 1.8‖
Normal	D2.GD	NT	32.4 ± 1.8	28.6 ± 1.7	29.7 ± 1.5
LCMV-immune	C3H.OL	NT	NT	55.3 ± 2.0‖	NT
LCMV-immune	B10.D2	62.7 ± 1.5‖	52.0 ± 1.7‖	72.7 ± 2.4‖	34.5 ± 2.4
Normal	B10.D2	30.5 ± 1.3	36.9 ± 1.4	32.2 ± 1.3	30.6 ± 1.8
LCMV-immune	BALB/c	56.1 ± 2.1‖	52.4 ± 1.3‖	47.9 ± 1.6‖	31.1 ± 1.4
LCMV-immune	C57BL/6	67.1 ± 1.6‖	36.5 ± 1.6	NT	75.0 ± 2.2‖
		Percent ^{51}Cr release from vaccinia-virus infected macrophages¶			
Vaccinia-immune**	D2.GD	NT	88.4 ± 1.8§‖	82.9 ± 2.1‖	71.3 ± 1.6‖
Normal	D2.GD	NT	53.2 ± 3.1	47.5 ± 2.6	43.8 ± 1.4
Vaccinia-immune	C3H.OL	NT	NT	90.5 ± 1.8‖	NT
Vaccinia-immune	B10.D2	NT	84.4 ± 2.5‖	84.8 ± 3.2‖	48.1 ± 2.0
Normal	B10.D2	NT	48.8 ± 1.9	48.1 ± 2.4	46.2 ± 1.7
Vaccinia-immune	BALB/c	NT	88.1 ± 2.0‖	96.0 ± 3.0‖	NT
Vaccinia-immune	C57BL/6	NT	NT	NT	101.5 ± 2.4‖

* Spleen cell:target cell ratio 30:1; incubation time 5 h at 37°C.

‡ Mice were infected with 2×10^3 i.c. LD_{50} of WE LCMV 7 days previously.

§ Means ± SEM of triplicates.

‖ Significantly different from release by normal spleen cells ($P < 0.05$).

¶ Tested at 30:1; incubation time 8 h at 37°C.

** Mice were infected with 2×10^7 PFU of WR vaccinia virus 6 days previously.

Virus Dose-Dependent Low Responsiveness of DBA/2 Mice is Recessive. The DBA/2 and D2.GD mice generated greatest cytotoxicity at the lowest infectious doses of LCMV. However, even under these conditions, BALB/c mice responded better than DBA/2 mice (Table VII). The results obtained with the established cell line J774 (*H-2*^{d}) as target were more clear-cut than those with C3H.OL macrophages. However, both results indicate that over a wide range of infectious doses tested 7 days after infection, BALB/c or C3H × DBA/2 F_1 mice responded with a least 10 times greater activity (Table VII). The low responsiveness is thus virus-dose dependent and recessive. This was further confirmed by the result that C3H and C3H × DBA/2 F_1 LCMV immune spleen cells were of comparable activity for LCMV-infected L cells.

Discussion

There is at present no conclusive evidence available for a role of *Ir* genes which determine susceptibility to virus infections in mice (10). The presented results also failed to reveal a demonstrable role for *I*-region genes in LCMV infections as far as generation of virus-specific cytotoxicity measured in vitro goes.

No LCMV or pox virus-specific cytotoxic activity associated with the *I* region of *H-2* could be detected under the conditions employed. *I*-region compatibility

TABLE VI
Low Cytolytic Activity Generated by LCMV-Infected DBA Mice

Spleen cells		Percent ^{51}Cr release from macrophage targets by spleen cells*							
		7 day LCMV immune‡				9 Day LCMV immune			
		C3H.OL		B10.A(4r)		C3H.OL		B10.A(4r)	
		Infected	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
D2.GD	1§	30.0 ± 2.1‖	28.7 ± 2.0	55.9 ± 2.5¶	34.2 ± 2.1	28.5 ± 1.0	26.7 ± 1.1	30.7 ± 1.2	29.1 ± 1.1
	2	33.1 ± 1.7	29.4 ± 1.2	59.8 ± 2.1¶	32.0 ± 1.3	25.9 ± 0.8	25.0 ± 2.0	33.3 ± 1.4	28.7 ± 0.9
	3	–	–	–	–	30.2 ± 1.2	28.3 ± 1.4	29.0 ± 1.1	28.1 ± 1.4
DBA/2	1§	27.8 ± 2.1	26.3 ± 2.1	30.1 ± 1.1	28.0 ± 1.7	26.2 ± 2.2	29.6 ± 1.1	29.8 ± 1.0	32.1 ± 2.1
	2	32.9 ± 1.5¶	27.5 ± 1.2	34.6 ± 1.4	31.3 ± 2.2	25.0 ± 2.5	28.0 ± 1.9	28.4 ± 2.1	27.4 ± 1.0
	3	–	–	–	–	22.7 ± 1.1	26.9 ± 0.8	26.6 ± 1.6	25.8 ± 2.7
B10.D2	1§	48.6 ± 2.0¶	28.9 ± 1.7	31.8 ± 1.4	32.8 ± 1.8	56.8 ± 2.5¶	28.3 ± 1.8	26.8 ± 1.5	25.0 ± 2.1
	2	45.1 ± 1.9¶	24.8 ± 2.3	34.6 ± 1.6	29.2 ± 1.6	52.0 ± 1.8¶	25.7 ± 2.2	28.5 ± 0.9	26.7 ± 1.1
	3	–	–	–	–	45.1 ± 3.1¶	29.5 ± 1.5	31.5 ± 1.1	28.2 ± 1.0
C57BL/10	1§	33.4 ± 1.2	30.3 ± 2.1	93.1 ± 2.8¶	33.2 ± 4.1	31.1 ± 1.0	32.8 ± 2.0	85.4 ± 3.1¶	30.7 ± 2.2
	2	30.8 ± 2.1	25.9 ± 2.4	82.3 ± 3.0¶	32.9 ± 2.0	32.0 ± 1.1	31.8 ± 0.7	79.5 ± 2.8¶	29.1 ± 1.8
Normal									
D2.GD		28.7 ± 2.1	28.1 ± 1.0	28.6 ± 2.1	26.7 ± 3.2				
DBA/2		27.2 ± 2.0	30.1 ± 2.1	27.7 ± 1.3	27.0 ± 1.0				
B10.D2		28.9 ± 1.1	28.5 ± 0.8	28.0 ± 1.0	26.1 ± 1.4				
C57BL/10		29.5 ± 2.2	28.1 ± 1.2	26.2 ± 2.4	25.7 ± 1.3				

* Immune spleen cells were assayed at 30:1 for 4 h at 37°C.
‡ Mice were infected i.v. with 2×10^6 i.c. LD_{50} 7 and 9 days previously.
§ Individual mice.
‖ Means ± SEM of triplicates.
¶ ^{51}Cr release significantly greater than relevant controls ($P < 0.05$).

alone did not result in any cytotoxicity, nor did it obviously facilitate *K*- or *D*-region-associated virus-specific activity (summarized in Table VIII). Nevertheless, minor activities could be easily missed because of the greater variability of macrophage targets as compared with established cell lines.

Macrophages have been shown to synthesize and to express (20–22) *Ia* structures which are coded for in the *I* region. Macrophages are members of the reticuloendothelial system and are probably the first cells to take up free virus from the circulation. Although it is not known as yet how and if cytotoxic T cells (24–28) play any direct role in controlling virus growth and/or spreading, interaction (cytolytic or noncytolytic in vivo) between T cells and infected macrophages are probably important for this function (reviewed in 29). These cells may thus represent biologically relevant targets. The conclusion that *I*-coded structures appear to be of certainly lesser relevance than *K*- or *D*-coded structures for virus-specific T-cell effector functions is supported by the finding that the adoptive transfer of both inflammatory process (18) and anti-viral protection is also dependent upon *K* or *D*, but not *I*-region compatibility (30).[2]

The capacity to generate D^d-associated cytotoxicity in vivo was also not under

[2] Zinkernagel, R. M., and R. M. Welsh. 1976. *H-2* compatibility requirement for virus-specific T-cell-mediated effector functions in vivo. I. Specificity of T cells conferring antiviral protection against lymphocytic choriomeningitis virus is associated with *H-2K* and *H-2D*. *J. Immunol.* Submitted for publication.

TABLE VII

Virus Dose-Dependent Low Responsiveness to LCMV Measured as Cytotoxic Activity is Recessive

Spleen cells	Dose of LCMV log 10 i.c. LD_{50}	Percent ^{51}Cr release from LCMV-infected targets*: ratio immune spleen cells: one target cell								
		J774			C3H.OL			L cells		
		30	10	3	30	10	3	30	10	3
BALB/c‡	1	80.6§‖	58.7‖	47.8	58.8‖	57.1‖	50.3	NT¶	NT	NT
	2	76.9‖	66.7‖	52.7	58.5‖	56.4‖	45.4	NT	NT	NT
	3	60.6‖	52.3‖	38.9	45.8	49.8	50.5	NT	NT	NT
	4	54.7‖	50.6‖	45.6	47.9	42.8	44.9	NT	NT	NT
D2.GD**	1	37.9	37.9	40.7	48.7	45.2	39.5	NT	NT	NT
	2	42.1	38.7	36.7	45.8	45.2	43.8	NT	NT	NT
	3	37.3	39.7	37.1	47.6	48.5	39.8	NT	NT	NT
	4	40.4	41.1	41.1	46.2	44.0	43.4	NT	NT	NT
DBA/2	1	52.9‖	49.6	46.1	61.4‖	55.9	54.1	NT	NT	NT
	2	49.2	50.5	44.5	47.9	52.1	46.8	NT	NT	NT
	3	44.9	44.3	40.0	57.6	48.9	48.8	NT	NT	NT
	4	41.5	35.7	36.2	42.7	42.7	44.6	NT	NT	NT
C3H × DBA/2 F_1	1	94.1‖	77.8‖	53.5	78‖	65‖	51	NT	NT	NT
	2	100.5‖	99.1‖	70.1	87‖	78‖	58‖	106.2‖	100.5‖	67.8‖
	3	95.7‖	100.9‖	69.8	99‖	67‖	65‖	96.7‖	90.2‖	59.5‖
	4	102.8‖	100.1‖	50.7	68‖	57‖	41	NT	NT	NT
C3H	1	NT	NT	NT	NT	NT	NT	NT	NT	NT
	2	NT	NT	NT	NT	NT	NT	86.2‖	90.4‖	55.6‖
	3	NT	NT	NT	NT	NT	NT	90.7‖	91.0‖	60.8‖
	4	NT	NT	NT	NT	NT	NT	NT	NT	NT
BALB	Normal	43.0	NT	NT	42.1	NT	NT	NT	NT	NT
D2.GD	Normal	42.2	NT	NT	46.3	NT	NT	NT	NT	NT
DBA/2	Normal	46.1	NT	NT	47.7	NT	NT	NT	NT	NT
C3H×DBA/2 F_1	Normal	48.7	NT	NT	50.8	NT	NT	33.5	NT	NT
C3H	Normal	NT	NT	NT	NT	NT	NT	28.2	NT	NT
Medium		45.6	NT	NT	43.2	NT	NT	26.0	NT	NT

* Spleen cells were assayed for 12 h at 37°C.

‡ Mice were injected i.v. with the indicated doses of LCMV 8 days previously. Results are from spleen cell pools of 2-3 mice.

§ Means of triplicates. SEM were from 0.5-1.5 for J774, 1.0-2.8 for C3H.OL, and 0.8-1.5 for L cells.

‖ Significantly ($P < 0.05$) greater than normal spleen cells on infected targets or immune cells on uninfected targets (data not shown).

¶ NT, not tested.

** D2.GD immune spleen cells from mice infected with 10 i.c. LD_{50} and assayed on infected C57BL/6 (H-2^b) targets gave lysis of 55% (30:1), 54% (10:1), and 45% (3:1); only the first two values being significantly greater than controls ($P < 0.05$); all other doses of infections did not cause generation of measurable cytotoxicity.

marked *I*-region control. The normal association between I^d and D^d in the *H-2*^{d} haplotype did not confer any greater potential to generate cytotoxicity than the other four or five *I*-region specificities tested in *H-2* recombinants. The semi-quantitative comparison of relative cytolytic activities did not vary more than a factor of 2.5, and mean activities per spleen were thus dictated mainly by the spleen cell numbers. Theoretically, the IC^k subregion of *H-2I* may have caused a lower response for LCMV-infected D^d targets as compared with K^d. Alternatively, the combination of IA^k–IC^k may generate lower responses than IA^k–IC^d;

TABLE VIII
Summary of the Genetic Analysis

Strain combinations tested (immune spleen cells-target)	*H-2* region compatibility					Lysis
	K	1		S	D	
		A	B C			
CBA/H-CBA/H, BALB/c-BALB/c C3H.OL-C3H.OL, D2.GD-D2.GD	—	—	—	—	—	+
BALB/c-C3H.OH, SJL-A.TH DBA/1-DA/HuSn	—	—	—	—		+
DBA/2-C3H.OL, B10.D2-C3H.OL C3H.OL-BALB/c	—	—	—			+
CBA/H-B10.A(2r)	—	—	—			+
BALB/c-D2.GD, C3H.OL-D2.GD D2.GD-C3H.OH, D2.GD-BALB/c	—	—				+
A.TL-SJL	—					+
A.TL-CBA/H		—	—	—		−
BALB/c-B10.A(2r)		—	—	—		−
D2.GD-C57BL/6		—	—	—	—	+
C57BL/6-B10.A(4r), D2.GD-B10.A(4r) B10.A-DBA/2, B10.A(5r)-DBA/2		—	—	—	—	+
C3H.OL-CBA/H				—	—	+
A.TL-BALB/c, BALB/c-A.TH, CBA/H-C3H.OH, SJL-DA/HuSn, C57BL/6-B10.A(2r), (A.TB, A.TD, A.TH, A.TL)-DBA/2					—	+

this explanation could be a possibility if a two gene model for *Ir* gene regulation of immune responsiveness is invoked (31, 32).

The variable, small differential activity to D^b, but not to K^d, in D2.GD mice could be explained in a similar way. The IA^d subregion by itself is not associated with low response to LCMV-altered K^d, e.g., in B10.D2 strong cytotoxicity against K^d-infected targets is observed. The combination of IA^d with IB^b and/or IC^b might thus impair generation of cytotoxicity. LCMV-immune B10.A(5r) are active for D^d targets, eliminating the possibility that the IB^b region by itself regulates low responsiveness. With the available mouse strains, it was not possible to distinguish whether IC^b alone or only in combination with IB^b and/or IA^d caused low response to K^d and D^b. But the small difference more likely reflects differentially greater reactivity to virus-altered D. Such examples have been previously described for LCMV-specific activity in C3H.OH mice for D^k and A strain mice for D^d targets (28, 33).

However, in view of the results presented in Tables IV–VII, the low responsiveness of D2.GD and DBA/2 mice, as compared with other *H-2*^{d} strains like BALB/c or B10.D2, is most likely explained by the influence of genes other than those of the *I*- or other *H-2*-gene regions. Different from *Ir*-controlled immune responses to soluble, defined antigens, low responsiveness to the WE strain of LCMV is inversely related to the infectious virus dose; high virus dose causes low responsiveness. But, as for the *Ir*-controlled mechanisms, low responsiveness is recessive (34). Low responsiveness in LCMV infections defined by the absence of detectable virus-specific cytotoxic activity in spleens may be caused by several mechanisms.

(a) Immune responsiveness may be regulated, in addition to *H-2*-coded *Ir* genes, by non *H-2*-coded ones (35–37). This study gave no direct evidence for such an argument.

(b) A more likely explanation is that gene(s) mapping outside the *H-2* gene complex control virus-growth characteristics. The low responsiveness could thus be only an apparent one and mainly due to an "adsorption" or "competition" phenomenon. LCMV is by itself not or only rarely cytopathogenic, and pathological processes are thus not generally caused by the virus itself (1–3). Extensive viral replication during a generalized infection could thus result in widespread tissue distribution of infected cells expressing LCMV-altered H-2K or D. Cytotoxic T cells could thus be "competed for" elsewhere in liver and other organs, or in spleens in mice containing many infected cells; such a mechanism (2) may well be the basis of the high dose immune paralysis phenomenon described by Hotchin (1) for acute LCM. High doses of LCMV injected i.c. may fail to cause fatal acute LCM. The LCMV strain used for the present studies is the viscerotropic WE strain, which apparently has less affinity to brain tissue than the neurotropic Armstrong strain of LCMV. WE LCMV, however, spreads much easier in spleen and liver. I.c. injection of Armstrong LCMV (100 i.c. LD_{50}) caused the death by classical LCM in DBA/2, as well as in BALB/c mice, while WE LCMV failed to do so with doses greater than 10 LD_{50} i.c. in DBA/2 mice, while BALB/c mice died of LCM with the same doses. These results are further evidence in favor of the interpretation that low activity may be a result of exhaustive recruitment of effector T cells away from the critical target organ, i.e., leptomeninges and brain (2, 7).

A similar non-*H-2*-linked differential capacity to deal with infectious virus has been found for ectromelia virus infections in mice (R. V. Blanden, B. D. Deak, and H. O. McDevitt, unpublished). A-strain mice are much more susceptible to subcutaneous ectromelia infection than C57BL/10 or C57BL/6 mice. However, both A and C57BL mice generate strong ectromelia-specific in vitro cytotoxicity when injected i.v. (17). Although a minor but significant factor of resistance is linked to *H-2*^{b}, non *H-2* genes appear to be of greater importance in determining susceptibility in this model. Also susceptibility to *Listeria monocytogenes* infection is greater for A than for C57BL/6 mice (R. M. Zinkernagel, unpublished observation), suggesting a possible involvement of macrophages in determining this trait.

Immune response to infectious viruses is associated predominantly with *K*- or *D*-coded structures, and no role for *I*-region genes could yet be demonstrated

regulating overall susceptibility or generation in vivo or activity in vitro of effector T cells. The discrepancies, with respect to specificity and probably regulation of immune responsiveness, between infectious agents and chemically defined, inert (i.e., not multiplying) antigens may well reflect fundamental biological differences. Soluble, noninfectious, well-defined antigens will, once injected, not increase in quantity. It is mainly the initial amount, the degree of toxicity, the rate of metabolic degradation, and immune elimination of these antigens which determines the quantity and quality of the immune response. Besides *Ir* genes, status of activation of macrophages, adjuvant effects, and cross-reactivity of antigens are probably amongst many other things, of importance in determining the nature of the immune response (38).

Viruses replicate within the host and reach maximal antigenic concentrations (e.g., virus antigens expressed on cell surfaces) within a few days. With these antigens, early and mainly nonimmunological mechanisms, which may control spread and multiplication of infectious agents, are possibly more important than *Ir* genes in determining the outcome of the developing protective immune response and the race between the damaging effect of infection, particularly in infections with cytopathogenic viruses. The sum of these factors sets the stage for the developing immune response. Immune regulatory mechanisms, like *Ir* genes, may further determine the response, but possibly only for antibody production. In addition, other immunological parameters, like degree of antigenic difference from host cell structures, may play a more crucial role. Viruses may resemble cell surface (e.g., *H-2*) structures and therefore escape immune attack by T-cell reactivity; alternatively failing to associate with *K* or *D* may result in escape from immune surveillance.

Summary

Lymphocytic choriomeningitis virus (LCMV) and ectromelia virus-specific T-cell-mediated cytotoxicity was assayed in various strain combinations using as targets peritoneal macrophages which have been shown to express Ia antigens. Virus-specific cytotoxicity was found only in *H-2K*- or *D*-region compatible combinations. *I*-region compatibility was not necessary nor alone sufficient for lysis. Six different *I*-region specificities had no obvious effect on the capacity to generate in vivo specific cytotoxicity (expressed in vitro) associated with D^d.

Low LCMV-specific cytotoxic activity generated in DBA/2 mice was caused by the non-*H-2* genetic background. This trait was inversely related to the infectious virus dose and recessive.

Non-*H-2* genes, possibly involved in controlling initial spread and multiplication of virus, seem to be, at least in the examples tested, more important in determining virus-specific cytotoxic T-cell activity in spleens than are *Ir* genes coded in *H-2*.

We thank Dr. Ada and Dr. F. Dixon for continuous support, Ms. G. Essery, Ms. N. Bowern, Ms. C. Woodham, and Ms. A. Althage for their enthusiastic technical assistance, and Mrs. J. Gouveia and Mrs. P. Minick for their help in preparing this manuscript.

Received for publication 22 April 1976.

References

1. Hotchin, J. 1971. Persistent and slow virus infections. *Virol. Mongr.* 3:1.
2. Doherty, P. C., and R. M. Zinkernagel. 1974. T cell-mediated immunopathology in viral infections. *Transplant. Rev.* 19:89.
3. Cole, G. A., and N. Nathanson. 1975. Lymphocytic choriomeningitis: pathogenesis. *Prog. Med. Virol.* 18:94.
4. Oldstone, M. B. A., and F. J. Dixon. 1970. Tissue injury in lymphocytic choriomeningitis viral infection: virus induced immunologically specific release of a cytotoxic factor from immune lymphoid cells. *Virology.* 42:805.
5. Marker, O., and M. Volkert. 1973. Studies on cell-mediated immunity to lymphocytic choriomeningitis virus in mice. *J. Exp. Med.* 137:1511.
6. Zinkernagel, R. M., and P. C. Doherty. 1974. Characteristics of the interaction in vitro between cytotoxic thymus-derived lymphocytes and target monolayers infected with lymphocytic choriomeningitis virus. *Scand. J. Immunol.* 3:287.
7. Doherty, P. C., R. M. Zinkernagel, and I. A. Ramshaw. 1974. Specificity and development of cytotoxic thymus-derived lymphocytes in lymphocytic choriomeningitis. *J. Immunol.* 112:1548.
8. Zinkernagel, R. M., and P. C. Doherty. 1973. Cytotoxic thymus-derived lymphocytes in cerebrospinal fluid of mice with lymphocytic choriomeningitis. *J. Exp. Med.* 138:1266.
9. Oldstone, M. B. A., F. J. Dixon, G. F. Mitchell, and H. O. McDevitt. 1973. Histocompatibility-linked genetic control of disease susceptibility. Murine lymphocytic choriomeningitis virus infection. *J. Exp. Med.* 137:1201.
10. Oldstone, M. B. A. (1976). Relationship between major histocompatibility antigens and disease: possible associations to human arenavirus diseases. *In* International Symposium on Arenaviral Infections of Public Health Importance, Atlanta, Ga. WHO Bull.
11. Zinkernagel, R. M., and P. C. Doherty. 1974. Restriction of in vitro lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature (Lond.).* 248:701.
12. Kindred, B., and D. C. Shreffler. 1972. H-2 dependence of cooperation between T and B cells *in vivo*. *J. Immunol.* 109:940.
13. Katz, D. H., M. G. Graves, M. E. Dort, H. Dimuzio, and B. Benacerraf. 1975. Cell interactions between histoincompatible T and B lymphocytes. VII. Cooperative responses between lymphocytes are controlled by genes in the *I* region of the *H-2* complex. *J. Exp. Med.* 141:263.
14. Bechtol, K. B., J. H. Freed, L. A. Herzenberg, and H. O. McDevitt. 1974. Genetic control of the antibody response to poly-L (Tyr, Glu)-poly-D,L-Ala-poly-L-Lys in C3H ↔ CWB tetraparental mice. *J. Exp. Med.* 140:1660.
15. Von Boehmer, H., L. Hudson, and J. Sprent. 1975. Collaboration of histoincompatible T and B lymphocytes using cells from tetraparental bone marrow chimeras. *J. Exp. Med.* 142:989.
16. Miller, J. F. A. P., M. A. Vadas, A. Whitelaw, and J. Gamble. 1975. H-2 gene complex restricts transfer of delayed-type hypersensitivity in mice. *Proc. Natl. Acad. Sci. U. S. A.* 72:5095.
17. Blanden, R. V., P. C. Doherty, M. B. C. Dunlop, I. D. Gardner, R. M. Zinkernagel, and C. S. David. 1975. Genes required for cytotoxicity against infected target cells in K and D regions of H-2 complex. *Nature (Lond.).* 254:269.
18. Doherty, P. C., R. V. Blanden, and R. M. Zinkernagel. 1976. Specificity of virus-

immune effector T cells for H-2K or H-2D compatible interactions: implications for H-antigen diversity. *Transplant. Rev.* **29**:89.

19. Zinkernagel, R. M. 1976. *H-2* compatibility requirement for virus-specific T-cell-mediated cytolysis. The *H-2K* structure involved is coded for by a single cistron defined by *H-2K^b* mutant mice. *J. Exp. Med.* **143**:437.
20. Unanue, E. R., M. E. Dorf, C. S. David, and B. Benacerraf. 1974. The presence of I region associated antigens on B cells in molecules distinct from immunoglobulin and H-2K and H-2D. *Proc. Natl. Acad. Sci. U. S. A.* **71**:5014.
21. Hammerling, G. J., G. Mauve, E. Goldberg, and H. O. McDevitt. 1974. Tissue distribution of Ia antigens. Evidence for Ia antigens on sperm cells and macrophages. *Immunogenetics.* **1**:428.
22. Delovitch, T. L., and H. O. McDevitt. 1975. Isolation and characterization of murine Ia antigens. *Immunogenetics.* **2**:39.
23. Shreffler, D. C., and C. S. David. 1975. The H-2 major histocompatibility complex and the I immune response region: genetic variation, function and organization. *Adv. Immunol.* **20**:125.
24. Cerottini, J. C., and K. T. Brunner. 1974. Cell-mediated cytotoxicity, allograft rejection and tumor immunity. *Adv. Immunol.* **18**:67.
25. Gardner, I., N. A. Bowern, and R. V. Blanden. 1974. Cell-mediated cytotoxicity against ectromelia virus-infected target cells. I. Specificity and kinetics. *Eur. J. Immunol.* **4**:63.
26. Koszinowski, U., and H. Ertl. 1975. Lysis mediated by T cells and restricted by H-2 antigen on target cells infected with vaccinia virus. *Nature (Lond.).* **255**:552.
27. Ralph, P., J. Pritchard, and M. Cohn. 1975. Reticulum cell sarcoma: an effector cell in antibody-dependent cell-mediated immunity. *J. Immunol.* **114**:898.
28. Doherty, P. C., and R. M. Zinkernagel. 1975. H-2 compatibility is required for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus. *J. Exp. Med.* **141**:502.
29. Blanden, R. V., A. J. Hapel, P. C. Doherty, and R. M. Zinkernagel. 1976. Lymphocyte-macrophage interactions and macrophage activation in the expression of antimicrobial immunity *in vivo*. *In* Immunobiology of the Macrophage, D. S. Nelson, editor. Academic Press, Inc., New York. 367.
30. Kees, U., and R. V. Blanden. 1976. A single genetic element in H-2K affects mouse T-cell antiviral function in pox virus infections. *J. Exp. Med.* **143**:450.
31. Dorf, M. E., J. H. Stimpfling, and B. Benacerraf. 1976. Requirement for two *H-2* complex *Ir* genes for the immune response to the GLØ terpolymer. *J. Exp. Med.* **141**:1459.
32. Merryman, C. F., P. H. Maurer, and J. H. Stimpfling. 1975. Unigenic and multigenic I region control of the immune response of mice to the GAT^{10} and GLØ-GLT terpolymers. *Immunogenetics.* **2**:441.
33. Zinkernagel, R. M., and P. C. Doherty. 1975. *H-2* compatibility requirement for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus. Different cytotoxic T-cell specificities are associated with structures coded for in *H-2K* or *H-2D*. *J. Exp. Med.* **141**:427.
34. Benacerraf, B., and H. O. McDevitt. 1972. Histocompatibility-linked immune response genes. *Science (Wash. D. C.).* **175**:273.
35. Mozes, E., H. O. McDevitt, J. C. Jaton, and M. Sela. 1969. The nature of the antigenic determinant in a genetic control of the antibody response. *J. Exp. Med.* **130**:493.
36. Silver, D. M., I. F. C. McKenzie, and H. J. Winn. 1972. Variations in the responses of

C57BL/10J and A/J mice to sheep red blood cells. I. Serological characterization and genetic analysis. *J. Exp. Med.* **136**:1063.

37. Dorf, M. E., E. K. Dunham, J. P. Johnson, and B. Benacerraf. 1974. Genetic control of the immune response: the effect of non-H-2 linked genes on antibody production. *J. Immunol.* **112**:1329.
38. Bretscher, P. A. 1974. On the control between cell-mediated IgM and IgG immunity. *Cell. Immunol.* **13**:171.