

SUSCEPTIBILITY TO LYMPHOCYTIC CHORIOMENINGITIS
VIRUS ISOLATES CORRELATES DIRECTLY WITH EARLY
AND HIGH CYTOTOXIC T CELL ACTIVITY, AS WELL AS
WITH FOOTPAD SWELLING REACTION, AND ALL THREE
ARE REGULATED BY H-2D

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Intracerebral (i.c.)¹ infection of mice with many isolates of lymphocytic choriomeningitis (LCM) virus (LCMV) causes in most mice a severe lethal choriomeningitis. The pathogenesis of this disease has been carefully studied for many years (1–5). Most investigators agree that T cells are crucially involved (1–7); some (7–10) argue in favor of cytotoxic T cells restricted to the major histocompatibility gene complex (MHC) class I antigens (H-2K and -D in mice) whereas others (11–14) interpret results to suggest that T cells involved in delayed-type hypersensitivity may be more instrumental. Since T cells that are restricted to H-2D class I but not class II H-2 antigens have been shown to adoptively transfer LCM-disease (8) as well as a primary type of footpad swelling reaction (15, 16) the two notions are not unreconcilable. In addition, it has been shown (17–19) in model situations in vitro that cytotoxic MHC class I-restricted T cells, as well as class II-restricted T cells, release a variety of soluble factors, e.g. interleukins, which may well trigger the delayed-type hypersensitivity like cellular infiltrates found in footpads or in leptomeninges.

We have compared two LCMV isolates, Docile (D) and Aggressive (A), (4, 20, 21) in a preliminary report (22), and present here more detailed experiments. The two LCMV isolates (4, 20, 22) caused either lethal LCM or no obvious disease, dependent upon the mouse strain tested. In all examples studied here, early and high cytotoxic T cell responses correlated with early and high primary footpad swelling reactions, and both directly correlated with great susceptibility to LCM disease. All three parameters were found to be predominantly regulated by the H-2D region.

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¹ *Abbreviations used in this paper:* A, aggressive strain of LCMV; D, docile strain of LCMV; i.c., intracerebral; i.f., in footpad; i.v., intravenous; LCM, lymphocytic choriomeningitis; LCMV, LCM virus; LU, lytic unit; MHC, major histocompatibility complex; NK, natural killer; pfu, plaque-forming unit.

Materials and Methods

Lymphocytic Choriomeningitis Virus. LCMV-A and LCMV-D of known concentration (measured in plaque-forming units [pfu]) were obtained directly from C. Pfau (14, 20, 21), Rensselaer Polytechnical Institute, Troy, NY. Stocks were diluted in medium containing 5% heat-inactivated fetal calf serum.

Mice. Mice were purchased from the Institute für Zuchthygiene, Abteilung Labortierkunde, Tierspital, Zürich (ICR, C57BL/6, BALB/c, DBA/2, C57BL/10, B10.BR, B10.D2, A/J, CBA/J, C3H/HeJ, and BALB.K), from OLAC, Bicester, Oxon, United Kingdom (B10.G, AKR, A2G, DBA/1, D2.GD), from The Jackson Laboratory, Bar Harbor, ME (B10.MBR), from Bantin-Kingman, Aldbrough, Hull, United Kingdom (SWR/J), or from Iffa-Credo, St. Germain-sur l'Arbresle, France (D2.GD, BALB.G, DBA/1, DBA/2). Some mice were a gift from the Institut für Biologisch-Medizinische Forschung AG, Füllinsdorf, Switzerland (C3H.Q), or the Basel Institute of Immunology, Basel, Switzerland (B6.AK1, B10.MBR). Mice of either sex were used at 8–20 wk of age.

Routes of Infection. Mice were injected either i.c. or into the footpad (i.f.) with 30 μ l, or intravenously (i.v.) with 0.2 ml of a virus dilution containing usually ~500–1,000 pfu of LCMV, unless stated otherwise.

Media. Minimal essential medium was supplemented with 5–10% heat-inactivated fetal calf serum, glutamine, bicarbonate, penicillin, and streptomycin. All ingredients were from KC-Biologicals, Lenexa, KS.

Cytotoxicity Assays. Activity of cytotoxic T cells was tested in ^{51}Cr -release assays (4, 23, 24). Target cells were established fibrosarcoma cell lines: MC57G (H-2^b), Q (H-2^a), D2 (H-2^d). The test was performed in round-bottomed 96-well plates. Effector cells and target cells (10^4) were each added in 100 μ l to give effector/target cell ratios of 40:1, 13:1, 4:1. They were mixed well and spun at ~1,000 g for 4 min. The test duration was usually 5 h. Spontaneous ^{51}Cr release is indicated in the Tables and Figures. To assess cytotoxic activity semiquantitatively, lytic units (LU) were determined according to established procedures (23) from dose-response curves; one LU represents the average number of spleen cells or meningeal infiltrate cells that lyse 33% of the target cells in a standard test well during the given test duration.

Meningeal Infiltrate Cells. The procedure used to obtain meningeal infiltrate cells has been described in detail previously (7, 8, 21, 25). Briefly, mice were ether-anesthetized and bled. The cerebrospinal fluid was tapped through the tectum of the fourth ventricle after careful preparation of the area. The skull cap was cut off, the brain and the skull were extensively rinsed with cold medium.

Primary Footpad Assay. Footpad swelling after local infection with LCMV (26, 27) was measured with a spring-loaded caliper (Kröplein, Schluchtern, Hessen, Federal Republic of Germany).

Results

Comparison of Various Methods for In Vivo Titration of LCMV-A vs. -D. Various doses of LCMV-A or -D (measured as pfu) were injected i.c. or i.f. into ICR outbred mice (Fig. 1). Mortality by day 12 caused by LCMV-A vs. -D injected i.c. was registered, and increase in footpad thickness 7 and 8 d after infection i.f. were assessed. The LD₅₀ for LCMV-A was ~2 pfu, whereas for LCMV-D it was ~0.2 pfu, confirming previous results by Pfau et al. (21). Footpad swelling was observed with ~3 pfu for LCMV-A, vs. ~1 pfu for LCMV-D. The mice that survived the i.c. infection were injected again on day 21 after primary infection with LCMV-Armstrong to determine whether protective immunity had developed. The mice that had been infected originally with ~1 pfu of LCMV-A or -D survived the second challenge. The 50% protective dose (PD₅₀) could be estimated at ~0.3 pfu for LCMV-A and ~0.2 pfu for LCMV-D. Thus, the LD₅₀ defined by mortality differed between A and D by a factor of 10–30 (as confirmed

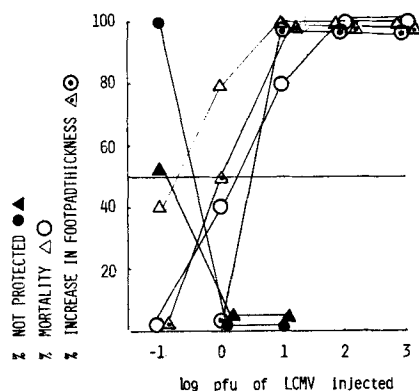


FIGURE 1. ICR outbred mice were injected i.c. or i.f. with 30 μ l containing various doses (pfu) of LCMV-A (○, ●, ◐) or -D (△, ▲, ◑). LCMV mortality (△, ○), incidence of >30% primary footpad swelling on day 7 and 8 (△, ●), or absence of protection against a second i.c. challenge by immunity induced during the first i.c. injection (▲, ●) were assessed. Averages of five mice per group.

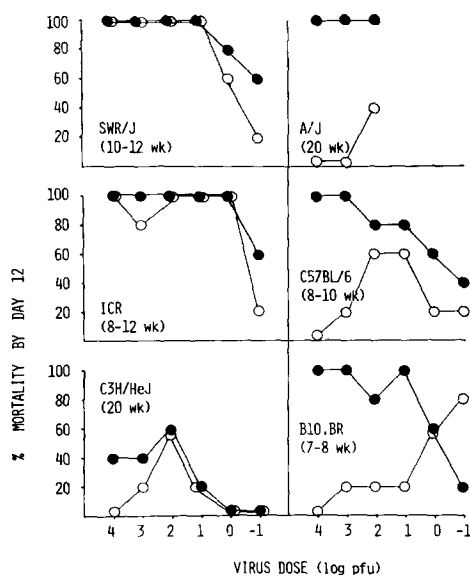


FIGURE 2. Mortality of various strains of mice injected i.c. with various doses (pfu) of LCMV-A (●) or -D (○).

also in two additional experiments). The doses needed to cause primary footpad swelling or to induce protective immunity in 50% of infected mice were quite comparable, and corresponded well with pfu titers.

Mortality Assessed in Various Mouse Strains. The susceptibility to LCMV-A- and -D-induced lethal disease varied considerably, dependent upon the mouse strains tested (Fig. 2). Percentage of mortality was comparable over a wide dose range in most strains (SWR/J, ICR, C57BL/6, A/J), but varied considerably in C3H/HeJ mice. Susceptibility to LCMV-induced lethal disease declined with doses of ≤ 1 pfu for LCMV-A or -D in most strains. Susceptibility to LCM

depended upon the LCMV-isolate and the mouse strain; e.g. in Fig. 2, SWR/J and ICR were highly susceptible to both LCMV-A- and -D-induced disease, whereas A/J, C57BL/6, and B10.BR were susceptible to LCMV-A, but resistant to -D.

A great variety of mouse strains were tested in many experiments for susceptibility to LCMV-A and -D as assessed by mortality and mean day of death (Table I). All possible phenotypes of disease susceptibility were found: susceptibility to both LCMV-A and -D (Type I, e.g. SWR/J); susceptibility to A, resistance to D (Type II, e.g. B10.BR); resistance to A, susceptibility to D (Type III, e.g. CBA/J); and resistance to both LCMV-A and -D (Type IV, e.g. DBA/2). Thus, susceptibility of mice to LCM disease depended upon the virus isolate used and upon the general genetic background of the host. LCMV-A caused lethal disease

TABLE I
Susceptibility to LCM Disease of Various Strains of Mice Infected i.c. with $\sim 5 \times 10^2$ pfu of LCMV

Exp.	Mouse strain	Background	H-2	LCMV injected				Phenotype
				A		D		
				Percent mortality	Mean day of death	Percent mortality	Mean day of death	
A	SWR/J	Swiss	q	100	7.5 ± 0.4	100	7.0 ± 0.2	I
	B10.G	B10	q	100	8.2 ± 0.3	100	8.1 ± 0.2	I
	C3H.Q	C3H	q	0		100	8.3 ± 0.3	III
	DBA/1	DBA	q	100	7.0 ± 0.8	100	8.7 ± 0.3	I
A	C3H/HeJ	C3H	k	20	9	80	10.2 ± 0.4	III
	AKR	AKR	k	100	8.1 ± 0.1	100	8.2 ± 0.2	I
	B10.BR	B10	k	100	8.8 ± 0.4	20	8	II
	CBA/J	CBA	k	40	8.5 ± 0.5	100	8	III
	BALB.K	BALB	k	100	9	0		II
	A/J	A	k/d	100	8	20	10	II
	A2G	A	k/d	100	7.5 ± 0.2	0		II
A	C57BL/10	B10	b	60	9.3 ± 0.6	20	10.5	II
	B10.BR	B10	k	100	8.8 ± 0.4	20	8	II
	B10.G	B10	q	100	8.2 ± 0.3	100	8.1 ± 0.2	I
A	C3H/HeJ	C3H	k	20	9	80	10.2 ± 0.4	III
	C3H.Q	C3H	q	0		100	8.3 ± 0.3	III
	DBA/1	DBA	q	100	7.0 ± 0.8	100	8.7 ± 0.3	I
	DBA/2	DBA	d	0		20	16	IV
B	BALB.B	BALB	b	100	8.1 ± 0.1	0		II
	BALB.K	BALB	k	100	9.0 ± 0.5	0		II
C	(SWR × DBA/2)	Swiss × DBA	q × d	100	9.0 ± 0.5	100	9.3 ± 0.5	I
	SWR	Swiss	q	100	7.2 ± 0.5	100	7.5 ± 0.6	I
	DBA/2	DBA	d	0		0		IV
D	CBA/J	CBA	k	0		100	7.5 ± 0.3	III
D	D2.GD	DBA	d/b	0		0		IV
	DBA/2	DBA	d	0		0		IV
	(BALB/c × DBA/2)F ₁	BALB × DBA	d	80	10.5 ± 0.5	0		II
	BALB/c	BALB	d	80	8.8 ± 0.5	0		II
	BALB.G	BALB	q	100	7.4 ± 0.3	0		II
E	(B10.BR × B10.AKM)F ₁	B10	k × k/q	100	8.5 ± 0.2	100	8.5 ± 0.3	I
	(B10.G × DBA/1)F ₁	B10 × DBA	q	90	8.7 ± 1.0	95	7	I
	(B10.BR × B10.G)F ₁	B10	k × q	100	8.0	100	7.5 ± 0.2	I

7-20-wk-old mice were injected i.c. with 30 μ l of LCMV. Results represent mean values of 5-10 mice per group; for H-2 type see ref. 28.

in BALB, most B10, AKR, A, DBA/1, and Swiss mice, but not, or only occasionally in CBA/J, C3H/HeJ, or DBA/2 mice. LCMV-D caused lethal LCM in Swiss (SWR/J and ICR), AKR, most CBA/J, DBA/1, and some C3H/HeJ, but not in B10, BALB, A, and DBA/2 mice. In most examples studied, the genetic background generally determined susceptibility. In many mouse strains tested where H-2 congenic mice were available, the H-2 did not influence susceptibility to LCM: BALB, CBA, C3H, and most B10 mice showed identical susceptibilities to LCMV-A or -D, independent of the H-2; e.g. BALB/c (H-2^d), BALB.B (H-2^b), BALB.K (H-2^k), and BALB.G (H-2^g) all were susceptible to LCMV-A and resistant to -D. However, in DBA and some B10 mice, susceptibility was influenced crucially by H-2 genes, in addition to virus and general genetic background. DBA/1 (H-2^q) were susceptible to both LCMV-A and -D, whereas DBA/2 (H-2^d) were resistant to both A and D. B10.G (H-2^g) mice were susceptible to LCMV-A and -D, whereas most other B10 H-2 congenic mouse strains were susceptible to A but resistant to D.

Although H-2^g seemed to confer increased susceptibility to LCMV-D (B10.G, SWR/J, DBA/1 all being H-2^g and susceptible to LCMV-D) this was not always the case, since BALB.G (H-2^g) were resistant to LCMV-D, and no significant difference in susceptibility to LCM was found when C3H/HeJ (H-2^k) and C3H.Q (H-2^q) mice were compared. F₁ mice were generally found to inherit disease susceptibility (Table I) e.g. (BALB/c × DBA/2)F₁ were susceptible to LCMV-A but resistant to -D, like BALB/c, but unlike DBA/2, which were resistant to both. Mean intervals between inoculation and death did not vary greatly. Overall, H-2^g mice died a little earlier (day 7–8) from LCMV-A than did other mice (day 7.5–9). Sometimes mice infected with LCMV-D virus died a little later than those infected with -A, e.g. C3H/HeJ, but the reverse was also found, e.g. (B10.G × DBA/1)F₁. In all examples studied, mice died of classical LCM with convulsions before day 11–12, but not thereafter, later deaths were caused by generalized wasting disease.

The results obtained so far indicate clearly that susceptibility to LCM disease depended upon at least three parameters: the LCMV isolate used (A vs. D), the general genetic background of the mice infected (Swiss mice, CBA, B10, DBA, etc.), and, in certain combinations tested, upon the H-2 genotype.

Cytotoxic T Cell Activity after i.v. Infection with LCMV-A and -D. Although somewhat controversial, many experiments (2–8) have suggested that susceptibility to LCM correlated well with the cytotoxic T cell activity generated in vivo. We tested several mouse strains representing different patterns of susceptibility to LCMV-A or -D. SWR/J mice were found to be highly susceptible to both A and D (Fig. 2). When infected i.v. with 5×10^2 pfu of either virus strain, they generated comparable cytotoxic T cell activities on day 6, 8, or 10 (Fig. 3). Lysis was comparable independent of the LCMV strain used to induce the T cell response, or that used for infecting Q (H-2^q) target cells. DBA/2 were resistant to both LCMV-A and -D (Table I), and failed to generate significant T cell activities after infection with either virus (Table II). C57BL/6 (H-2^b) and B10.D2 (H-2^d) mice were susceptible to LCMV-A and resistant to -D (Table I). B10.D2 mice generated measurable cytolytic activity when infected with LCMV-A but not with -D (Table II). When C57BL/6 mice were infected i.v. with $\sim 5 \times 10^2$

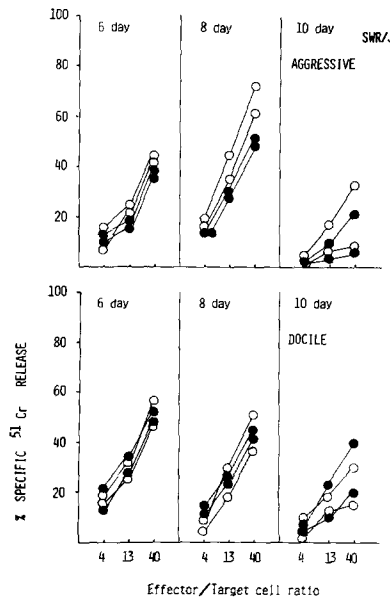


FIGURE 3. Two SWR/J (H-2^a) mice, 8-12 wk of age, were infected i.v. with LCMV-A (*top*) or -D (*bottom*), and tested individually on Q (H-2^a) target cells infected with A (●) or D (○). Test duration, 4 h, spontaneous release: Q-LCMV-A, 17%; Q-LCMV-D, 19%; uninfected Q targets were lysed <5%.

TABLE II
LCMV-specific Cytotoxic T Cell Activity in DBA/2 and B10.D2 Mice on Day 8

Mouse strain	LCMV strain	LU/spleen	
		D2 LCMV	D2 uninfected
B10.D2	A	800-2,000	<20
	D	<20	<20
DBA/2	A	<20	<20
	D	<20	<20

2-4 mice per group were injected i.v. with 5×10^2 pfu of LCMV 8 d previously. LU were measured on D2 targets infected with LCMV-A or -D, with comparable results. Test duration was 5 h, spontaneous release <17%.

pfu of LCMV-A or -D, the kinetics shown in Fig. 4 and Table III were found for natural killer (NK) and cytotoxic T lymphocyte activities. NK activity was comparable in C57BL/6 mice infected with A and with D (Fig. 4); it seemed to persist slightly longer, albeit with lower activity, after infection with LCMV-A. Cytotoxic T cell activity of LCMV-A- or -D-infected C57BL/6 mice was tested on MC57G (H-2^b) targets infected with A or with D; both targets were lysed to comparable extents regardless of the virus isolate used for infecting target cells (Fig. 4). However, activity rose more quickly, peaked higher, and persisted longer in mice infected with LCMV-A than with -D (Fig. 4). In a separate experiment, when LU per spleen were calculated on day 7 and day 9, the

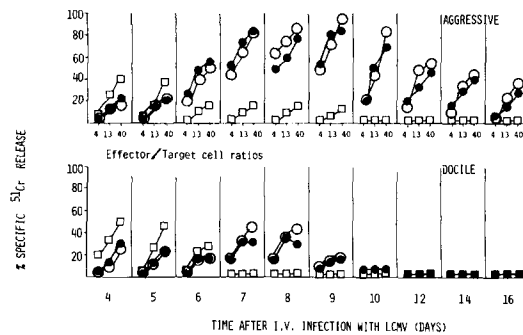


FIGURE 4. C57BL/6 mice, 7–9 wk of age, were injected i.v. with 0.2 ml containing ~1,000 pfu of LCMV-A (top) or -D (bottom). Results of two individual mice tested on MC57G (H-2^b) target cells infected with LCMV-A (●) or -D (○), or on YAC (□) cells. Test duration 6 h, effector/target ratios: 40:1, 13:1, 4:1; spontaneous release for MC57G-A, 18%; MC57G-D, 21%; YAC, 14%. (■) Indicates that no significant ⁵¹Cr release was measured on all targets tested.

TABLE III
Cytolytic Activity in Spleens of C57BL/6 Mice Infected i.v. with Various Doses of LCMV-A or -D

LCMV strain	LU ($\times 10^{-2}$) per spleen at LCMV doses (pfu) of:							
	5		50		500		5,000	
	Day 7	Day 9	Day 7	Day 9	Day 7	Day 9	Day 7	Day 9
A	4	230	18	110	25	180	27	30
D	3	85	15	0.3	3	0.4	1	<0.6

Effector cells were tested at various ratios in a 5-h ⁵¹Cr-release assay on LCMV-A- and -D-infected MC57G (H-2^b) target cells; LU were determined from the dose-response curves. Lysis of NK-sensitive YAC targets was <10 LU/spleen. Spontaneous release on infected targets was 17–20%; on uninfected targets, 21%.

differences became more obvious (Table III). With 5×10^1 – 5×10^3 pfu of LCMV-A, C57BL/6 generated 1,800–2,700 LU/spleen, vs. 300–600 LU/spleen after infection with LCMV-D. On day 9, LCMV-A-infected C57BL/6 had 11,000–23,000 LU/spleen, but -D-infected mice had <100 LU/spleen. With the highest dose of LCMV-A tested, cytotoxic T cell activity in spleens on day 9 was relatively low, i.e. 3,000 LU, and not higher than on day 7; this lower T cell response is a common finding in mice infected with high doses of LCMV (4, 29). With low doses of LCMV-A and -D, responses were low on day 7, then responses with LCMV-A rose, but were only two to three times higher than for LCMV-D on day 9 (Table III); this relatively small difference fits with the finding that, with low doses of LCMV-D, some C57BL/6 died (Fig. 2).

Cytotoxic T Lymphocyte Activity after i.c. Infection with LCMV-A vs. -D. We tested representative strains of mice for their capacity to generate LCMV-specific cytotoxic T cells in spleens and leptomenigeal infiltrates after i.c. infection with LCMV-A or -D. SWR/J mice, which are susceptible to both LCMV-A and -D, generated comparably high lytic activity in meningeal infiltrates and spleens when tested on day 6 (Table IV). Later LU could not be tested, since all mice had died by day 7. C57BL/6 mice that are susceptible to LCMV-A and resistant

TABLE IV
LCMV-specific Cytotoxic T Cell Activity in SWR/J Mice Infected i.c. with LCMV

LCMV strain	Meningeal infiltrate				Spleen	
	CSF* cells ($\times 10^{-2}$)		LU [‡]		LU ($\times 10^{-2}$)	
	Day 5	Day 6	Day 5	Day 6	Day 5	Day 6
A	30	110	<1	52	2	150 [§]
D	35	120	<1	30	12	110

Mice were injected i.c. with 10^8 pfu of LCMV; all mice injected 7 d previously died.

* Cerebrospinal fluid (CSF) was taken and cells per microliter were counted.

[‡] Test duration 6 h, spontaneous release from LCMV-D-infected (H-2^g) target cells was 21%; <1 LU/spleen was found on uninfected targets.

[§] Effector cells from day 6 were also tested on LCMV-A-infected target cells (20% spontaneous release) with 140 vs. 95 LU for LCMV-A vs. LCMV-D immune spleen cells.

TABLE V
Cytolytic T Cell Activity in C57BL/6 Mice Infected i.c. with LCMV-A or -D

Virus dose (pfu)	LU ($\times 10^{-2}$) per spleen on day:								
	5		6		7		8		
	A	D	A	D	A	D	A	D	
2,000	<1	2	24	6	55	26	45	8	
200	<1	<1	16	7	115	18	37	16	
20	<1	<1	9	6	12	8	40	55	
	LU per meningeal infiltrate:								
	2,000	<1	<1	30	1	250	40	270	30
	200	<1	<1	10	3	270	20	280	250
	20	<1	<1	3	1	30	4	80	ND

Mice were injected with 30μ l containing various amounts of LCMV. Cytotoxicity was assessed in a 5-h test with various effector/target cell ratios, and LU were calculated from dose-response curves. Spontaneous release on (MC57G-LCMV-D) infected target cells was 25%. ND, not determined.

to -D were injected with various doses of A or D i.c., and their spleen cells and meningeal infiltrate cells were assayed for cytotoxic T cell activity on day 5, 6, 7, and 8 (Table V). Clearly, LU per meningeal infiltrates were higher in LCMV-A-injected mice on all days tested, with differences being particularly great on days 6 and 7; diminishing on day 8. The same trend was seen in spleens of the same mice, but with smaller differences than found for meningeal infiltrate cells. When a lower infectious dose of 20 pfu was used, lytic activity (LU) in meningeal infiltrates and spleens of D-infected mice increased quite considerably on day 8, and mice did occasionally die of these doses (see Fig. 2).

H-2D-linked Susceptibility to LCMV-D in B10 Mice. H-2 congenic B10 mice show consistent susceptibility to LCMV-A (Table I). As shown in a preliminary report (22), we confirmed here that B10 mice possessing the H-2D^a allele were susceptible to LCMV-D (Table VI). C57BL/10 (H-2K^b, I^b, D^b), B10.BR (K^k, I^k,

TABLE VI
H-2^q-linked Susceptibility to LCMV-A vs. -D in B10 Background Mice

Strain	Haplotype H-2KID	Percent mortality		Mean day of death	
		A	D	A	D
B10.MBR	bkq	100	100	7.0	8.1 ± 0.9
B10.BR	kkk	100	0	7.9 ± 0.1	Survived*
B6.AK1	bbk	100	0	8.0	Survived
C57BL/10	bbb	100	0	8.1 ± 0.3	Survived
B10.G	qqq	100	100	8.2 ± 0.4	8.0 ± 0.4

5–10 mice were injected i.c. with 30 μ l containing 10³ pfu of LCMV-A or -D.

* Observation period, 20 d.

TABLE VII
Dose Dependence of Primary Footpad Swelling Caused by LCMV-A and -D on Day 7 Postinfection

Mouse strain	Virus strain	Percent specific increase of footpad thickness in mice given doses (pfu) of:		
		50	500	5,000
SWR/J	A	125	100	105
SWR/J	D	105	115	80
C57BL/6	A	<5	110	100
C57BL/6	D	<5	20	<5

Average of measurements of 4–8 footpads; SEM <10%.

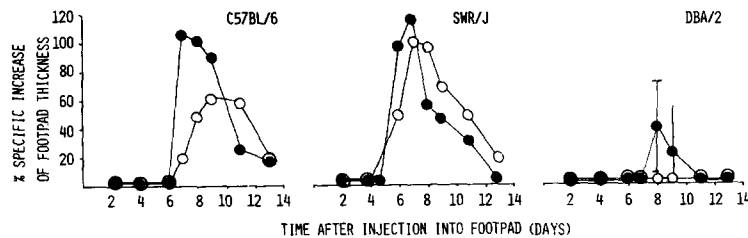


FIGURE 5. Mice were injected with 10³ pfu of LCMV-A (●) or -D (○) into the footpad. Footpad thickness was measured daily thereafter and compared with preinjection values. Values are means of measurements of 6–8 footpads; SEM <10% of the values indicated for SWR/J and C57BL/6, and are not shown. Range of values varied greatly for DBA/2 mice, and therefore are shown.

D^k), and B6.AK1 (K^b, I^b, D^k) were all resistant to 10³ pfu of LCMV-D i.c., whereas B10.MBR (K^b, I^k, D^q) and B10.G (K^q, I^q, D^q) were susceptible, thereby mapping susceptibility to LCMV-D to H-2D^q.

Primary Footpad Swelling Reaction after Local Infection with LCMV-A vs. -D. The primary footpad swelling reaction after local infection with LCMV is strictly T cell-dependent (15, 26, 27). In SWR/J mice, which are susceptible to both LCMV-A and -D, the kinetics of the local footpad response to 5 × 10² pfu of LCMV-A or -D was virtually identical (Table VII and Fig. 5). In C57BL/6 mice,

which are susceptible to LCMV-A and resistant to -D, the footpad reaction to A increased rapidly from 0–110% swelling between day 6 and day 7, but D-infected mice showed much lower and slower responses (Fig. 5). The comparable responses in A- or D-infected SWR/J, and the differential responses in C57BL/6 mice were seen over a wide range of infectious doses (Table VII). The response to small doses (50 pfu) in C57BL/6 only became measurable on day 8 after infection, and then showed values of 80% swelling for A vs. 10% for D (not shown). In both strains of mice, footpad swelling declined a little slower in D- than in A-infected mice (Fig. 5).

DBA/2 mice, which were resistant to both LCMV isolates, developed virtually no or only variable primary footpad swelling upon infection with 5×10^2 pfu of either A or D (Fig. 5).

Footpad Swelling Reaction Is Regulated by H-2D. After local infection into the footpad with LCMV-A vs. -D, early footpad swelling was measured on day 6 in various H-2 congenic mouse strains. Pronounced differences were seen, which correlated with susceptibility to LCM after i.c. inoculation (Table VIII). Footpad measurements on day 8 and day 9 revealed smaller differences (see Fig. 5, C57BL/6). When B10 congenic mice were injected simultaneously i.c. and i.f., the following results were obtained: all B10 mice showed rapid and early footpad swelling after infection with LCMV-A, and all mice died of LCM by day 8–9. The same mice showed a differential response after i.c. and local infection into the footpad with LCMV-D (Fig. 6). Whereas B10.G (K^q , I^q , D^q) and B10.AKM (K^k , I^k , D^q) mice showed a rapid and high response, B10.A (K^k , $I^{k/d}$, D^d) and B10.BR (K^k , I^k , D^k) mice showed variably lower and slower responses. B10.G (K^q , I^q , D^q) and B10.AKM (K^k , I^k , D^q) died on days 8 and 9; B10.A (K^k , $I^{k/d}$, D^d)

TABLE VIII
Primary Footpad Swelling on Day 6 vs. Lethal LCM
Immunopathology

Mouse strain (H-2)	LCMV strain	Percent increase of footpad thickness on day 6*	Positive of total mice injected*	Percent mortality of LCM [‡]
DBA/1 (q) [§]	A	70 ± 5	5/5	100
	D	21 ± 4	4/5	100
DBA/2 (d)	A	5	0/5	0
	D	5	0/5	0
B10.G (q)	A	60 ± 3	5/5	100
	D	49 ± 6	5/5	100
B10.BR (k)	A	43 ± 8	5/5	100
	D	8 ± 5	2/5	0

* Mice were injected i.f. with 1,000 pfu of LCMV.

[‡] Mice were injected i.c. with 1,000 pfu of LCMV.

[§] 3–5 mice were injected into one footpad; swelling was compared with the uninfected footpad.

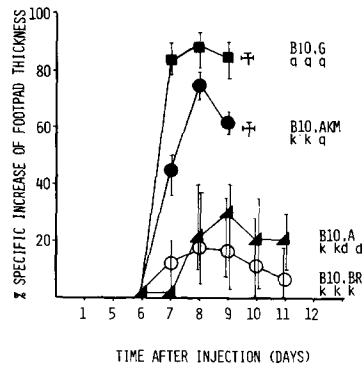


FIGURE 6. Various B10 congenic mice were simultaneously injected with 1,000 pfu i.c. and i.f. of LCMV-D. Percent increase of footpad thickness was measured daily. The values indicate the averages of 6–8 footpads; the ranges are also shown.

and B10.BR (K^k , I^k , D^k) mice survived. Since B10.AKM differ genetically from B10.BR mice only in the H-2D region, both susceptibility to LCMV-D and the capacity to mount a rapid and high footpad swelling reaction were shown to be regulated by H-2D; D^q conferring high responsiveness, and D^k causing lower responsiveness.

Discussion

Two plaque variants of LCMV isolated from a carrier mouse (20, 21) showed the following characteristics *in vivo*: depending upon both the virus isolate injected i.c. and the strain of mice tested, the susceptibility to LCM disease varied greatly, most drastically with non-H-2 background genes. Four phenotypes of disease susceptibility patterns were found in young adult mice (6–20 wk): susceptibility to both, resistance to both, susceptibility to one LCMV isolate and resistance to the other, and vice versa. However, in certain combinations of mouse strain and LCMV isolate, a dominant regulatory role of H-2 became apparent. As reported elsewhere (22) and documented here with DBA mice and additional B10 H-2-recombinant mouse strains, susceptibility to LCMV-D could be shown, in a few examples, to be crucially regulated by H-2, in B10 mice particularly by H-2D. These results are compatible with those from recent studies (30) with BALB/c and D^{dm} mutant mice, which showed that mice carrying the D^{dm2} mutations were less susceptible to the LCMV-Armstrong isolate; only 50% of i.c.-infected D^{dm2} mice died, as compared to 100% for BALB/c, which have the wild type D^d .

All three parameters studied here, i.e. susceptibility to LCM, cytolytic T cell activity (22), and primary footpad swelling are shown to be regulated by H-2D. This correlation indicates that both cytolytic activity *in vitro* (and possibly *in vivo*) and recruitment of inflammatory cells is mediated by the same T cell subpopulation, restricted predominantly to class I H-2D-coded antigens (4). This may settle the dispute as to whether delayed-type hypersensitivity mechanisms (11–13) rather than cytotoxic T cells are responsible for LCM disease (7–10); it appears likely that the same effector T cells are responsible for both phenomena. This and earlier results (15, 16) also indicate that delayed-type hypersensitivity

can be induced by MHC class I- or class II-restricted T cells (24); since both types of T cells release various lymphokines involved in recruitment of inflammatory cells, this finding is not unexpected. In general, antigens that actively (or by fusion) induce new antigenic determinants on cell surfaces (such as viruses) induce T cells (including those mediating delayed-type hypersensitivity) that are restricted to class I or class II MHC products; antigens that become passively associated with cell surfaces (such as antigens of intracellular bacteria or soluble antigens) trigger T cells specific for class II MHC products (24).

Non-MHC background genes influenced susceptibility to LCM drastically. Outbred ICR and SWR/J (Swiss) mice were, in general, susceptible to both LCMV isolates. BALB, C57BL, and A mice were susceptible to LCMV-A and resistant to -D; C3H/HeJ and CBA/J mice tested were more susceptible to D than to A. Differences in susceptibility to LCM or in the capacities of various strains to generate cytotoxic T cells have been noticed previously: outbred Swiss mice or inbred mice derived from them have repeatedly been found (31-33) to be highly susceptible to LCM. DBA/2 mice have been shown (34) to be low responders to LCMV-WE. DBA/1 and C57BL/6 mice were found (31, 33) to be high responders to LCMV-WE, while AKR and CBA/J were shown (31-33) to be low responders when tested for their cytotoxic T cell activities as well as primary footpad reaction.

The results obtained with C3H/HeJ mice differ from published data (14, 21). Pfau et al. routinely used C3HeB/FeJ mice 3-4 wk of age, and found that these mice were clearly susceptible to LCMV-A and resistant to -D. Since C3H and CBA mice bred in different laboratories vary greatly (35), these results may be a reflection of such genetic differences (33). Our data show a very clear correlation between susceptibility to LCM and the capacity to mount an early and high LCMV-specific cytotoxic T cell response. In C57BL/6 mice, which are susceptible to LCMV-A, i.c. or i.v. infection with this LCMV isolate induced high cytolytic activities in meningeal infiltrates and spleen; in contrast, LCMV-D caused no or only occasional deaths, and induced 5-10 times lower cytolytic activities, which rose more slowly (Tables III and V, and Fig. 4). If infected with relatively low doses of LCMV-D, C57BL/6 mice generated considerable levels of cytotoxic T cells, and occasionally died of LCM (see also Fig. 2). This result is compatible with the explanation that LCM disease develops only if effector T cells are recruited preferentially to the brain. Since LCMV-D replicates more rapidly than LCMV-A (21), -D usually spreads widely, and effector cells may be diverted to sites other than the brain, inducing systemic nonlethal disease (2, 4, 21, 29, 36-39). However, with a small dose of LCMV-D, the brain remains the main target organ and the disease may be lethal. Both the relatively short interval during which lethal LCM may develop (i.e. dependent upon mouse strain during a 2-3-d interval starting on day 6-8), and the virus dose dependence (2, 36) show that lethal LCM is the result of a delicate balance between virus localization and spread, and immune T cell response (36-39). This complex biological equilibrium resulting in susceptibility or resistance to lethal LCM disease can therefore not be an all-or-none difference with respect to cytotoxic T cell activities or primary footpad swelling; 5-10-fold differences in relative cytotoxic activities and 1-2-d differences in the respective kinetics are apparently respon-

sible for great differences in susceptibility to LCM disease. Studying C3HeB/FeJ mice on day 8 after i.c. infection with ~500 pfu of LCMV-A or -D, Pfau et al. (14) found no correlation between susceptibility to LCM and cytotoxic T cell activity found in spleens or meningeal infiltrates. As documented here, monitoring a single timepoint may not reveal the differing kinetics of the antiviral response that, herein, correlated clearly with susceptibility. In fact, an infectious dose of LCMV-A and -D comparable to the one used by Pfau et al. (2×10^2 pfu) initiated generation of equal activities of cytotoxic T cells in C57BL/6 mice when tested on day 8 (Table V); however they differed vastly on days 6 and 7. Therefore, it is to be expected that the cytotoxic T cell response of C3HeB/FeJ mice will correlate with susceptibility to LCM when tested with various LCMV doses over several days.

MHC association of LCM disease susceptibility has first been documented by Oldstone et al. (32); however, these findings have not been confirmed (40, 41). Our previous report (22) and this study of LCMV-D in B10 mice, as well as the results obtained with mutant D^{dm} mice and LCMV-Armstrong (30), show susceptibility as mapping to H-2D. The reported (40, 41) difficulties to confirm H-2-dependence of LCM susceptibility in C3H mice may be due to many experimental variables such as genetic variation (35), age of mice, and substrain of LCMV used. In addition, the possibility that the health status of experimental mice may influence susceptibility to LCM must be further evaluated.

The observed differences in susceptibilities to LCM are difficult to explain. Obviously, the A vs. D pattern depends upon both LCMV isolate and mouse strain. Since all possible susceptibility patterns have been observed, no easy explanation can be given. Differences in virus replication (21) and spread, organotropism (2, 3), α , β , or γ interferon induction and/or susceptibility to it, (30, 42–44), as well as differences in antigenicity and/or immunogenicity (22, 30, 45–47) may all be invoked to explain the findings. As pointed out above, differential LCMV replications and spreads correlate with LCM disease (2, 21, 37). The rapid replication and wide spread of the virus generally correlated with D, whereas slow and restricted growth characterized the A pattern. These parameters are currently being studied for the mouse strains studied here; but in B10 mice the above general rule that LCM-D replicates faster than LCMV-A has been confirmed in a preliminary experiment. Published results on interferon levels in LCMV infections do not allow any conclusion as to the respective role of α , β , or γ interferon in causing the D or A pattern (43). Although not serologically distinguished so far, LCMV isolates may differ with respect to antigens defined by monoclonal antibodies as shown for rabies virus (45) and/or recognized by T cells. Ahmed et al. (46) and Byrne et al. (47) have found differences among various LCMV strains when assayed for their capacity to serve, on infected cells, as target antigens for cytotoxic T cells. These differences may cause induction of differing T cell responses mainly dependent upon MHC Ir genes. Such possible antigenic differences are now being searched for in LCMV-A and -D; they could readily explain why, in B10 mice, H-2D influences susceptibility to D but not to A. A surprising conclusion is that complex antigens such as viruses appear to induce only very few, or most probably only one antigen(s) or antigenic site(s) recognized by biologically relevant effector T cells,

otherwise MHC regulation of an antiviral T cell immune response could not become so obviously limiting.

In conclusion, LCMV-induced disease is a model in mice exhibiting clear susceptibility differences varying with virus isolate, general genetic background, and major transplantation antigens of the host. MHC disease associations are recognized only when a particular virus isolate infects the appropriate mouse strain. In many respects, this model reflects the situation found in outbred humans (48, 49). MHC disease associations are relatively weak and variable. This may probably be due to: (a) the possibility that known or unknown non- or poorly cytopathic infectious agents that seem to be serologically identical may differ antigenically as seen by T cells; (b) many important non-HLA genes vary throughout the population and amongst different races, and (c) serologically defined HLA types may actually comprise several variant HLA molecules (24, 50–52). In this study, inbred mice and cloned virus isolates reduce these numerous variables to show that, under well-defined conditions, MHC disease association is very strong and may directly reveal MHC-regulated T cell-mediated immunopathology.

Summary

The lymphocytic choriomeningitis virus (LCMV) isolates Docile (D) and Aggressive (A) of Pfau et al. (21) were studied in various strains of mice. Disease susceptibility, assessed as mortality and time to death to LCMV-D or -A varied greatly amongst mouse strains, and all four possible susceptibility patterns were observed: susceptibility to both (e.g. SWR/J), resistance to both (e.g. DBA/2), susceptibility to A but resistance to D (C57BL/6), or vice versa (CBA/J). Irrespective of the virus isolate or the mouse strain tested, susceptibility correlated with both early and high cytotoxic T cell activity found in spleens or leptomeningeal infiltrates, and with early and high primary footpad swelling reaction after local infection. C57BL/6 mice infected with A or SWR/J infected with A or with D showed, in both test systems, early and high activities; in contrast, DBA/2 mice infected with either D or A, and C57BL/6 infected with D showed no or only slow and low responses in both tests. Early and high LCMV-specific cytotoxic T cell activity, and the rapidity and extent of the primary footpad reaction directly correlated with susceptibility to LCM and all were dominantly regulated by H-2D.

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