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Virus Specificity of Cytotoxic T Lymphocytes Generated During Acute Lymphocytic Choriomeningitis Virus Infection: Role of the *H-2* Region in Determining Cross-Reactivity for Different Lymphocytic Choriomeningitis Virus Strains†

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We have compared the relatedness of five different strains of lymphocytic choriomeningitis virus (LCMV) as assessed by LCMV-specific cytotoxic T lymphocytes (CTL). Several different mouse strains were injected with each of the five LCMV strains, and the cross-reactivity of virus-specific CTL generated during the acute infection was tested by killing on a panel of target cells infected with the various LCMV strains. We found that the cross-reactivity pattern of LCMV-specific CTL generated in mice of *H-2*^{d} haplotype (BALB/c WEHI and DBA/2) was strikingly different from that in mice of *H-2*^{b} haplotype (C57BL/6 and C3H.Sw/Sn), suggesting that the fine specificity of LCMV-specific CTL is a function of the *H-2* region. The characteristic cross-reactivity patterns were also observed in (C57BL/6 × DBA/2)F1 mice, demonstrating that the repertoire of the *H-2*^{b}- and *H-2*^{d}-restricted LCMV-specific CTL is not changed as a result of complementation by gene products of the other major histocompatibility haplotype. Studies with congenic BALB.B10 and (BALB.B10 × BALB/c)F1 mice firmly established that the characteristic cross-reactivity patterns of LCMV-specific CTL map to the *H-2* region and are not influenced by background genes outside the major histocompatibility locus. These results suggest that LCMV determinants seen in the context of *H-2*^{d}-restricting elements are different from those seen in the context of *H-2*^{b}-restricting elements. Moreover, our studies show that CTL can be used as probes for dissecting differences among various LCMV strains, but the degree of relatedness between the different LCMV strains is not absolute when measured by CTL recognition. Since the *H-2* region regulates the fine specificity of CTL generated during LCMV infection in its natural host, the degree of cross-protective immunity developed during a viral infection apparently depends on the major histocompatibility haplotype. The importance of these findings lies in understanding susceptibility or resistance of various host populations to viral infections and in designing vaccination programs to provide immunity.

Cell-mediated immunity is an important factor in the control and elimination of several viral infections (6, 16, 31, 41). One manifestation of the cell-mediated immune response is the generation of cytotoxic T lymphocytes (CTL) that kill virus-infected cells, thereby curtailing virus production and limiting the spread of infection. Such CTL show specificity for the virus and for self-determinants encoded by the *K* or *D* end of the major histocompatibility (MHC) complex (41). Several studies have shown that these *H-2*-restricted CTL play a crucial role in protection against viral infection (1, 16, 24, 41).

Infection of mice with lymphocytic choriomeningitis virus (LCMV) is an excellent experimental model for studying the generation, specificity, and biological role of CTL induced during viral infection in the natural host. In fact, the *H-2* restriction of virus-specific CTL was first demonstrated with LCMV (40). Other studies have shown that LCMV-specific CTL play an important role in recovery from acute infection (28, 43). The observation that mice persistently infected with LCMV contain no detectable LCMV-specific CTL suggests an association between their absence and the inability to clear the infection (12, 25). LCMV-specific CTL have also been implicated in the immunopathological process causing lethal acute choriomeningitis after intracerebral inoculation of adult mice with LCMV (11, 14). Thus, the CTL response clearly has important biological consequences in LCMV infection.

LCMV is the prototype virus of the *Arenavirus* group (10). The genome of LCMV consists of two segments of single-stranded RNA of negative polarity (32). The large segment, designated L, has a molecular weight (MW) of ca. 2×10^6, and the small segment, designated S, has an MW of ca. 1×10^6. Three major viral structural polypeptides have been identified: an internal nucleoprotein (MW, 63,000) associated with the genomic RNA and two surface glycoproteins, GP-1 (MW, 43,000) and GP-2 (MW, 36,000). GP-1 is the predominant viral polypeptide expressed on the surface of infected cells (M. J. Buchmeier, *in* R. Compans and D. Bishop, ed., *Negative Strand Viruses*, in press). Although there is only one serotype of LCMV, several individual LCMV strains can be distinguished by oligonucleotide T1 mapping, tryptic peptide mapping, and monoclonal antibody analysis (8, 9, 15). These LCMV strains vary considerably in their ability to cause tissue injury and disease (15).

As part of our long-term objective to define the molecular basis of LCMV pathogenesis, we analyze here the interaction between CTL and LCMV-infected cells. We have examined the degree of relatedness among five strains of LCMV, Armstrong, WE, UBC, Traub, and Pasteur, as assessed by the cross-reactivity of CTL generated during acute infection with each LCMV strain in mice of distinct *H-2* types. We found that the cross-reactivity pattern of LCMV-specific CTL generated in mice of *H-2*^{d} haplotype is

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TABLE 1. Fine specificity of LCMV-specific cytotoxic T cells generated in BALB/c WEHI (*H-2*^{d}) mice

Effectors[a]	E/T[b]	% Specific ^{51}Cr release from BALB Cl-7 (*H-2*^{d}) targets[c]					
		Uninfected	Armstrong	Pasteur	WE	UBC	Traub
BALB/c-uninfected	12.5	1	4	0	2	2	2
	25	5	10	1	3	4	3
	50	7	12	3	7	6	6
BALB/c-Armstrong	12.5	1	59	8	53	16	60
	25	3	69	15	63	25	74
	50	8	79	23	70	33	80
BALB/c-Pasteur	12.5	0	45	27	42	37	42
	25	1	56	33	56	48	57
	50	2	63	42	66	55	66
BALB/c-WE	12.5	0	16	0	18	1	16
	25	0	29	0	25	0	25
	50	0	53	0	30	3	30
BALB/c-UBC	12.5	0	15	3	16	24	22
	25	0	20	4	22	37	33
	50	0	22	9	31	49	40
BALB/c-Traub	12.5	0	37	0	18	6	43
	25	2	45	0	24	9	44
	50	3	59	0	47	7	55

[a] Spleen cells from BALB/c WEHI mice infected i.p. 8 days previously with 2×10^5 PFU of the indicated LCMV strain. Three mice were infected with each LCMV strain, and the spleen cells were pooled for the assay.
[b] Effector/target cell ratio.
[c] Test duration was 6 to 8 h. Spontaneous release was 15 to 40%. All samples were assayed in triplicate, and the standard error was usually <2%.

completely different from that seen in *H-2*^{b} mice. Our results indicate that the nature of cross-protective immunity developed during a viral infection depends on the MHC haplotype. This genetic control of virus-specific CTL responses has important implications in the use of vaccines to provide immunity against viral infections.

MATERIALS AND METHODS

Mice. BALB/c WEHI (*H-2*^{d}), C57BL/6 (*H-2*^{b}), DBA/2 (*H-2*^{d}), C3H.Sw/Sn (*H-2*^{b}), (C57BL/6 × DBA/2)F1 (*H-2*^{b} × *H-2*^{d}), BALB.B10 (*H-2*^{b}), and (BALB.B10 × BALB/c)F1 (*H-2*^{b} × *H-2*^{d}) mice were obtained from the breeding colony at Scripps Clinic and Research Foundation, La Jolla, Calif. All mice used in these experiments were 6 to 12 weeks of age. BALB.B10 (*H-2*^{b}) mice are congenic with BALB/c (*H-2*^{d}) mice, differing only at the *H-2* region; i.e., the former are BALB background but with *H-2*^{b} haplotype.

Virus. The origins of the five LCMV strains used in the study have been described previously (15). All virus strains were triple plaque purified on Vero cells, and then stocks were grown in BHK-21 cells. Virus stocks at passage 1 or passage 2 levels were used in all experiments.

Generation of primary CTL response in vivo. Mice were infected intraperitoneally (i.p.) with 2×10^5 PFU of the indicated LCMV strain. Eight days postinfection the mice were sacrificed, spleens were harvested, single-cell suspensions were made, and lymphoid cells were tested for the presence of LCMV-specific CTL.

Cytotoxicity assay. Single-cell suspensions of spleens, free of erythrocytes, were prepared in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, antibiotics, and L-glutamine. These cells were tested for cytotoxicity against a panel of target cells infected with one of the LCMV strains at a multiplicity of infection of 0.5 and used 18 to 24 h later (BALB Cl-7 [*H-2*^{d}] or B.10.D2 [*H-2*^{d}]) or 48 h later (MC-57 [*H-2*^{b}]). The target cells were labeled with ^{51}Cr, and the cytotoxicity assay was performed as previously described (43; J. A. Byrne, R. Ahmed, and M. B. A. Oldstone, J. Immunol., in press). Percent specific ^{51}Cr release (each release was measured in counts per minute) was calculated by the formula: [(sample release − spontaneous release)/(maximum release − spontaneous release)] × 100.

RESULTS

Cross-reactivity of LCMV-specific CTL generated in BALB/c WEHI (*H-2*^{d}) mice. Separate groups of BALB/c WEHI mice were injected i.p. with each of the five LCMV strains, and the cross-reactivity of LCMV-specific CTL generated subsequently was tested by killing of syngeneic target cells (BALB Cl-7 or B.10.D2) infected with the various LCMV strains. A similar pattern of killing was observed with either BALB Cl-7 (*H-2*^{d}) or B.10.D2 (*H-2*^{d}) targets. Several experiments were done in BALB/c WEHI mice, and the pattern of cross-reactivity shown by LCMV-specific CTL was highly consistent. The results of one of five experiments are shown in Table 1. CTL generated after infection with the Armstrong strain killed targets infected with Armstrong, WE, and Traub strains of LCMV efficiently, but killed Pasteur- and UBC-infected targets less effectively. This pattern of killing was most striking in the CTL populations induced by WE and Traub LCMV strains in that killing of Pasteur- and UBC-infected targets was minimum or nonexistent, but targets infected with the other three LCMV strains (Armstrong, WE, and Traub) were efficiently lysed. Pasteur-induced CTL were cross-reactive and killed targets infected with all five LCMV strains. CTL generated after infection with UBC showed reduced killing of Pasteur-infected targets and, in some experiments, also killed Armstrong targets inefficiently. LCMV-infected allogeneic (*H-2*^{b}) targets and Pichinde virus-infected syngeneic (*H-2*^{d})

targets were included in some experiments, and there was no killing of these targets, showing that the killing of LCMV-infected syngeneic ($H\text{-}2^d$) targets was due to *H-2*-restricted LCMV-specific CTL (data not shown).

The following conclusions can be made from the experiments done with BALB/c WEHI mice. (i) CTL induced by Armstrong, WE, and Traub strains of LCMV are totally cross-reactive and kill targets infected with any of these strains; thus, these three LCMV strains are closely related on the basis of CTL cross-reactivity. (ii) Pasteur and UBC strains of LCMV are easily distinguishable from the other three LCMV strains. CTL induced by WE and Traub (and to a lesser extent Armstrong) do not recognize targets infected with UBC or Pasteur. However, this distinction is unidirectional, since CTL generated during infection with UBC and Pasteur kill WE-, Traub-, and Armstrong-infected targets. These results suggest that all five LCMV strains share certain common determinants seen by CTL and that there are other determinants recognized by LCMV-specific CTL that are present in Armstrong, WE, and Traub but are absent in Pasteur and UBC. Furthermore, the "unique" determinants present in WE and Traub are immunodominant; infection with WE or Traub induces a population of anti-LCMV CTL that does not kill Pasteur- or UBC-infected targets. (iii) Pasteur and UBC strains can also be distinguished from each other by UBC-generated CTL, suggesting that viral determinants seen by CTL in UBC-infected cells are lacking in Pasteur-infected cells.

Cross-reactivity of LCMV-specific CTL generated in C57BL/6 ($H\text{-}2^b$) mice. The results of one of three experiments done in C57BL/6 mice are shown in Table 2. The CTL populations induced after infection with any of the five LCMV strains were cross-reactive and killed targets infected with any LCMV strain. There was somewhat reduced killing of WE targets by Pasteur- or UBC-induced CTL, but this was not seen consistently in every experiment. The patterns of cross-reactivity shown by LCMV-specific CTL from C57BL/6 mice and BALB/c WEHI mice were strikingly different, as is most evident when one compares results with WE and Traub infections in BALB/c WEHI and C57BL/6 mice (cf. Tables 1 and 2). Infection of BALB/c WEHI ($H\text{-}2^d$) mice with WE or Traub generated a population of LCMV-specific CTL that did not kill Pasteur- or UBC-infected targets. In contrast; C57BL/6 ($H\text{-}2^b$) mice infected with the same viruses (WE and Traub) generated LCMV-specific CTL that efficiently killed Pasteur and UBC targets. These results show that the degree of relationship between the different LCMV strains as measured by CTL recognition is not absolute but varies depending on the strain of mouse used to generate the CTL.

Cross-reactivity of LCMV-specific CTL generated in DBA/2 ($H\text{-}2^d$) and C3H.Sw/Sn ($H\text{-}2^b$) mice. To further check the influence of the *H-2* region on these cross-reactivity patterns, we examined the fine specificity of LCMV-specific CTL generated in two additional mouse strains. The virus specificity of CTL generated in DBA/2 ($H\text{-}2^d$) mice is shown in Table 3. The pattern of cross-reactivity against the different LCMV strains resembled that in BALB/c WEHI ($H\text{-}2^d$) mice (Table 1). Thus, both mouse strains with the $H\text{-}2^d$ haplotype generated LCMV-specific CTL with similar cross-reactivity patterns. The virus specificity of CTL generated in C3H.Sw/Sn ($H\text{-}2^b$) (Table 4) mice differed from that seen in DBA/2 and BALB/c WEHI mice but was similar to the pattern seen in C57BL/6 ($H\text{-}2^b$) mice (cf. with Table 2). These results strongly suggest that the cross-reactivity pattern of LCMV-specific CTL is a function of the MHC genes.

Cross-reactivity of LCMV-specific CTL generated in (C57BL/6 × DBA/2)F1 ($H\text{-}2^b \times H\text{-}2^d$) mice. F1 ($H\text{-}2^b \times H\text{-}2^d$) mice were infected with each of the five LCMV strains, and the cross-reactivity of LCMV-specific

TABLE 2. Fine specificity of LCMV-specific cytotoxic T cells generated in C57BL/6 ($H\text{-}2^b$) mice

Effectors[a]	E/T[b]	% Specific ^{51}Cr release from MC-57 ($H\text{-}2^b$) targets[c]					
		Uninfected	Armstrong	Pasteur	WE	UBC	Traub
C57BL/6-uninfected	12.5	0	1	0	0	1	2
	25	0	1	2	1	2	3
	50	1	3	4	4	4	5
C57BL/6-Armstrong	12.5	0	39	39	30	47	39
	25	0	58	60	48	71	58
	50	0	73	77	65	86	79
C57BL/6-Pasteur	12.5	0	19	30	12	30	21
	25	0	32	48	20	47	36
	50	0	48	63	30	65	53
C57BL/6-WE	12.5	0	14	21	8	14	12
	25	0	20	29	16	29	21
	50	0	41	46	25	50	44
C57BL/6-UBC	12.5	0	6	12	4	10	6
	25	0	15	20	9	17	14
	50	0	29	37	16	37	26
C57BL/6-Traub	12.5	0	52	41	32	40	31
	25	0	61	49	39	57	39
	50	0	73	79	57	71	68

[a] Spleen cells from C57BL/6 mice infected i.p. 8 days previously with 2×10^5 PFU of the indicated LCMV strain. There were two mice in each group, and spleen cells were pooled for the assay.

[b] Effector/target cell ratio.

[c] Test duration was 6 to 8 h. Spontaneous release was <15%. All samples were assayed in triplicate, and the standard error was usually <2%.

TABLE 3. Fine specificity of LCMV-specific cytotoxic T cells generated in DBA/2 (*H-2*^{d}) mice

Effectors[a]	E/T[b]	% Specific ^{51}Cr release from BALB Cl-7 (*H-2*^{d}) targets[c]					
		Uninfected	Armstrong	Pasteur	WE	UBC	Traub
DBA/2-uninfected	12.5	2	4	8	3	3	1
	25	3	6	10	8	7	0
	50	7	9	14	12	12	3
DBA/2-Armstrong	12.5	4	66	25	59	24	60
	25	4	77	41	74	38	67
	50	3	77	40	75	44	70
DBA/2-Pasteur	12.5	2	28	20	20	21	15
	25	4	54	34	36	36	29
	50	4	59	39	45	40	40
DBA/2-WE	12.5	NT[d]	31	0	29	10	17
	25	2	49	0	31	0	21
	50	4	58	0	43	4	31
DBA/2-UBC	12.5	NT	15	15	22	22	9
	25	3	33	20	21	31	15
	50	3	40	19	31	40	24
DBA/2-Traub	12.5	0	32	2	20	3	16
	25	2	50	7	35	9	31
	50	1	64	8	43	12	42

[a] Spleen cells from DBA/2 mice infected i.p. 8 days previously with 2×10^5 PFU of the indicated LCMV strain. There were two mice in each group, and spleen cells were pooled for the assay.
[b] Effector/target cell ratio.
[c] Test duration was 6 to 8 h. Spontaneous release was 20 to 40%. All samples were assayed in triplicate, and the standard error was <2%.
[d] NT, Not tested.

CTL was tested on a panel of *H-2*^{b} and *H-2*^{d} targets infected with the various LCMV strains. The *H-2*^{d}- and *H-2*^{b}-restricted CTL responses of these F1 mice had different cross-reactivity patterns (Table 5). Moreover, the *H-2*^{d}-restricted CTL cross-reactivity pattern was similar to the pattern seen in BALB/c WEHI (*H-2*^{d}) (Table 1) and DBA/2 (*H-2*^{d}) (Table 3) mice, whereas the *H-2*^{b}-restricted CTL response of F1 mice had a cross-reactivity pattern similar to that seen in C57BL/6 (*H-2*^{b}) (Table 2) and C3H.Sw/Sn (*H-2*^{b}) (Table 4) mice. These results provide further evidence that the *H-2* region plays an important role in determining the virus specificity of LCMV-specific cytotoxic T cells. The experiments in F1 mice also show that the "repertoire" of the *H-2*^{b}- and *H-2*^{d}-restricted LCMV-specific CTL is not changed as a result of complementation by gene products of the other MHC haplotype.

TABLE 4. Fine specificity of LCMV-specific cytotoxic T cells generated in C3H.Sw/Sn (*H-2*^{b}) mice

Effectors[a]	E/T[b]	% Specific ^{51}Cr release from MC-57 (*H-2*^{b}) targets[c]					
		Uninfected	Armstrong	Pasteur	WE	UBC	Traub
C3H.Sw/Sn-uninfected	12.5	0	0	0	0	0	1
	25	1	0	0	2	0	0
	50	1	0	0	5	0	1
C3H.Sw/Sn-Armstrong	12.5	0	28	27	15	23	16
	25	0	44	42	22	36	28
	50	1	61	60	36	52	42
C3H.Sw/Sn-Pasteur	12.5	2	33	39	9	30	21
	25	2	50	56	12	42	34
	50	2	61	64	22	57	42
C3H.Sw/Sn-WE	12.5	1	14	13	5	13	10
	25	1	25	22	13	23	19
	50	0	36	33	20	33	27
C3H.Sw/Sn-Traub	12.5	2	47	47	36	43	50
	25	2	69	63	51	65	66
	50	3	75	69	62	76	76

[a] Spleen cells from C3H.Sw/Sn mice infected i.p. 8 days previously with 2×10^5 PFU of the indicated LCMV strain. There were two mice in each group, and spleen cells were pooled for the assay.
[b] Effector/target cell ratio.
[c] Test duration was 6 to 8 h. Spontaneous release was <15%. All samples were assayed in triplicate, and the standard error was usually <2%.

Cross-reactivity of LCMV-specific CTL generated in congenic mice. To confirm that the different cross-reactivity patterns shown by LCMV-specific CTL in *H-2*^{d} and *H-2*^{b} mice were due to the MHC region and not due to genetic loci outside of the MHC region, we tested the LCMV-specific CTL responses of congenic BALB.B10 (*H-2*^{b}) and (BALB.B10 × BALB/c)F1 (*H-2*^{b} × *H-2*^{d}) mice. BALB.B10 (*H-2*^{b}) mice contain all the background genes of BALB/c mice except the MHC region, which is derived from C57BL/10 mice. Thus, these are essentially BALB mice but with an *H-2*^{b} haplotype. The CTL response of BALB.B10 mice after infection with Armstrong, WE, and Traub strains is shown in Table 6 (experiments 2 and 3). Armstrong-induced CTL killed targets infected with all five LCMV strains tested. Killing of Pasteur- and UBC-infected targets was especially good in contrast to the cross-reactivity pattern seen in BALB/c (*H-2*^{d}) mice (cf. Tables 1 and 6). Similarly, Traub-induced CTL from BALB.B10 (*H-2*^{b}) mice efficiently killed Pasteur- and UBC-infected targets. Also, WE-induced CTL were cross-reactive and killed targets infected with any of the five LCMV strains. It should be noted that CTL generated in BALB/c (*H-2*^{d}) mice after Traub or WE infection showed minimal or no lysis of Pasteur and UBC targets (Table 1). Thus, the *H-2*^{b}-restricted CTL response of these congenic mice showed a cross-reactivity pattern similar to that seen in the earlier experiments with mice of the *H-2*^{b} haplotype. The one exception was the reduced killing of Armstrong targets by Traub-induced CTL. Nevertheless, the overall cross-reactivity pattern of LCMV-specific CTL generated in BALB.B10 (*H-2*^{b}) mice (Table 6) closely resembled the pattern seen in C57BL/6 (*H-2*^{b}) and C3H.Sw/Sn (*H-2*^{b}) mice (Tables 2 and 4) and differed strikingly from the cross-reactivity pattern seen in BALB/c (*H-2*^{d}) and DBA/2 (*H-2*^{d}) mice (Tables 1 and 3). We also tested the CTL response of (BALB.B10 × BALB/c)F1 congenic mice (Table 6, experiment 1). Once again, the *H-2*^{b}-restricted CTL response showed a different cross-reactivity pattern than the *H-2*^{d}-restricted CTL response. Moreover, the *H-2*^{b}-restricted LCMV-specific CTL showed a cross-reactivity pattern similar to that seen earlier in *H-2*^{b} mice, and the *H-2*^{d}-restricted CTL response was similar to the cross-reactivity pattern seen in *H-2*^{d} mice. These results firmly establish that the fine specificity of virus recognition by LCMV-specific CTL is determined by the gene products of the *H-2* region.

DISCUSSION

We have examined the virus specificity of primary CTL generated during acute LCMV infection in several different strains of mice. The major finding of this study is that the cross-reactivity of LCMV-specific CTL for different LCMV strains is determined by the gene products of the *H-2* region. In the summary of our overall results, which Table 7 illustrates, the cross-reactivity pattern of *H-2*^{d}-restricted LCMV-specific CTL is clearly different from that of *H-2*^{b}-restricted LCMV-specific CTL. Studies with congenic mice show unequivocally that the characteristic cross-reactivity patterns shown by LCMV-specific CTL map to the *H-2* region and are not influenced by background genes outside the MHC locus. Thus, our results indicate that the *H-2* region determines the degree of cross-protective immunity generated during a viral infection. This observation has important implications in the design of vaccination programs to provide immunity against viral infections, because the degree of cross-protective immunity generated in vaccinated individuals may vary depending on their MHC haplotype.

We have compared the relatedness of five LCMV strains as assessed by LCMV-specific CTL. In mice of *H-2*^{d} haplotype, the Armstrong, WE, and Traub strains of LCMV induced CTL populations that were mutually cross-reactive. Thus, these three LCMV strains are closely related on the basis of recognition by *H-2*^{d}-restricted LCMV-specific CTL. However, the *H-2*^{d}-restricted CTL induced by WE and Traub (and to a lesser extent Armstrong) strains of LCMV did not kill targets infected with UBC or Pasteur strains. This lack of cross-reactivity was unidirectional since UBC- and Pasteur-induced CTL lysed WE-, Traub-, and Armstrong-infected targets. These results suggest that all five

TABLE 5. Fine specificity of LCMV-specific cytotoxic T cells generated in (C57BL/6 × DBA/2)F1 (*H-2*^{b} × *H-2*^{d}) mice

Effectors[a]	E/T[b]	% Specific ^{51}Cr release from BALB Cl-7 (*H-2*^{d}) targets[c]						% Specific ^{51}Cr release from MC-57 (*H-2*^{b}) targets[c]					
		Uninfected	Armstrong	Pasteur	WE	UBC	Traub	Uninfected	Armstrong	Pasteur	WE	UBC	Traub
(B6 × D2)F1-uninfected	16	0	5	3	1	2	0	0	2	1	0	2	1
	50	7	11	6	9	9	6	0	3	5	1	2	0
(B6 × D2)F1-Armstrong	16	7	72	17	61	29	71	2	46	57	27	53	40
	50	6	72	20	66	29	74	2	72	85	46	70	56
(B6 × D2)F1-Pasteur	16	0	40	17	19	29	25	1	45	62	24	48	36
	50	0	45	27	26	40	33	3	68	85	39	73	56
(B6 × D2)F1-WE	16	0	49	0	27	4	45	2	25	29	12	34	22
	50	0	60	4	34	8	56	1	47	53	23	57	42
(B6 × D2)F1-UBC	16	0	26	8	15	16	27	2	48	46	32	54	51
	50	0	50	10	31	25	40	2	63	77	50	72	64
(B6 × D2)F1-Traub	16	0	68	0	58	16	70	2	58	64	33	59	50
	50	0	69	2	50	25	73	3	75	93	52	72	67

[a] Spleen cells from (C57BL/6 × DBA/2)F1 mice infected i.p. 8 days previously with 2 × 10^5 PFU of the indicated LCMV strain. There were two mice in each group, and spleen cells were pooled for the assay.

[b] Effector/target cell ratio.

[c] Test duration was 6 to 8 h. Spontaneous release was 20 to 40% from BALB Cl-7 (*H-2*^{d}) targets and <15% from MC-57 (*H-2*^{b}) targets. All samples were assayed in triplicate, and the standard error was usually < 2%.

TABLE 6. Fine specificity of LCMV-specific cytotoxic T cells generated in congenic BALB.B10 (*H-2*^{b}) and (BALB.B10 × BALB/c)F1 (*H-2*^{b} × *H-2*^{d}) mice

Expt no.	Effectors[a]	E/T[b]	% Specific ^{51}Cr release from MC-57 (*H-2*^{b}) targets[c]						% Specific ^{51}Cr release from BALB Cl-7 (*H-2*^{d}) targets[c]				
			Uninfected	Armstrong	Pasteur	UBC	Traub	WE	Uninfected	Armstrong	Pasteur	UBC	Traub
1	(BALB.B10 × BALB/c)F1-Armstrong	16	2	50	47	49	40	NT[d]	8	71	31	41	79
		50	0	62	60	60	50	NT	5	71	19	33	82
	(BALB.B10 × BALB/c)F1-WE	16	0	9	7	10	2	NT	1	31	1	0	19
		50	0	19	16	18	7	NT	1	46	1	0	23
	(BALB.B10 × BALB/c)F1-Traub	16	0	26	22	24	23	NT	4	53	15	13	60
		50	0	41	33	35	33	NT	2	61	5	8	61
2	BALB.B10-Armstrong	16	4	37	73	62	36	NT					
		50	10	60	100	81	61	NT					
	BALB.B10-WE	16	1	10	14	10	5	NT					
		50	7	14	24	17	11	NT					
	BALB.B10-Traub	16	6	7	35	27	15	NT					
		50	10	16	56	45	31	NT					
3	BALB.B10-Armstrong	16	2	67	59	64	49	61					
		50	5	77	70	78	72	84					
	BALB.B10-WE	16	2	42	48	48	30	47					
		50	4	67	63	70	54	67					

[a] Spleen cells from BALB.B10 or (BALB.B10 × BALB/c)F1 mice infected i.p. 8 days previously with 2 × 10^5 PFU of the indicated strain. There were two mice in each group, and spleen cells were pooled for the assay.
[b] Effector/target cell ratio.
[c] Test duration was 6 to 8 h. Spontaneous release was 20 to 40% from BALB Cl-7 (*H-2*^{d}) targets and <15% from MC-57 (*H-2*^{b}) targets. All samples were assayed in triplicate, and the standard error was usually <2%.
[d] NT, Not tested.

LCMV strains share certain common determinants seen by *H-2*^{d}-restricted CTL and that additional unique determinants present in Armstrong, WE, and Traub LCMV strains are absent in Pasteur and UBC. Although all five LCMV strains share common determinants, the unique determinants present in Traub and WE are immunodominant since WE- or Traub-induced CTL do not kill Pasteur- or UBC-infected targets. In contrast to the pattern seen in *H-2*^{d} mice, the LCMV-specific CTL response in *H-2*^{b} mice was totally cross-reactive. Although quantitative differences were noted in some experiments, in general, *H-2*^{b}-restricted LCMV-specific CTL induced by any of the five LCMV strains killed targets infected with any LCMV strain. Recent studies by Byrne et al. (in press) examining the cross-reactivity of LCMV-specific CTL clones extend the observations we make here at the population level. All of 11 *H-2*^{b}-restricted LCMV-specific CTL clones induced by the Armstrong strain killed targets infected with each of the five LCMV strains, whereas 1 *H-2*^{d}-restricted CTL clone induced by Armstrong killed Armstrong-, Traub-, and WE-infected targets but not Pasteur or UBC targets (Byrne et al., in press). Thus, the characteristic cross-reactivity pattern of *H-2*^{b}- and *H-2*^{d}-restricted LCMV-specific CTL that we have described at the population level is also seen at the clonal level.

Two additional points should be emphasized. First, CTL can be used as probes for dissecting differences among various LCMV strains. These studies should lead to a classification of LCMV strains and perhaps shed light on their evolution. The second and more crucial point is that the degree of relatedness between the different LCMV strains is

TABLE 7. Cross-reactivity patterns of *H-2*^{d}- and *H-2*^{b}-restricted LCMV-specific CTL

LCMV strain used to induce CTL	Killing of targets infected with the following strain[a]:									
	Armstrong		Pasteur		WE		UBC		Traub	
	H-2^d	*H-2*^{b}	*H-2*^{d}	*H-2*^{b}	*H-2*^{d}	*H-2*^{b}	*H-2*^{d}	*H-2*^{b}	*H-2*^{d}	*H-2*^{b}
Armstrong	+	+	↓	+	+	+	↓	+	+	+
Pasteur	+	+	+	+	+	±	+	+	+	+
WE	+	+	↓ ↓	+	+	+	↓ ↓	+	+	±
UBC	±	+	↓	+	+	±	+	+	+	+
Traub	+	±	↓ ↓	+	+	+	↓ ↓	+	+	+

[a] Symbols: +, efficient killing of targets; ±, somewhat reduced killing but not consistently seen in every experiment; ↓, reduced killing in all experiments; ↓ ↓, marginal or no killing in all experiments.

not absolute when measured by CTL recognition but is influenced by the MHC gene products of the responder mice. Consequently, data regarding CTL with respect to a single *H-2* haplotype cannot be generalized to other *H-2* haplotypes. For example, WE and Traub strains of LCMV induced a CTL population in *H-2*^{d} mice that did not recognize targets infected with Pasteur or UBC strains of LCMV, but the same LCMV strains (WE and Traub) induced a CTL population in *H-2*^{b}mice that efficiently killed targets infected with Pasteur or UBC. These results suggest that the LCMV determinants seen in the context of *H-2*^{d}-restricting elements are different from those seen in the context of *H-2*^{b}-restricting elements.

The cross-reactivity of virus-specific CTL has been examined in several other virus systems (35, 42). Both CTL that are specific only for the inducing virus and CTL that are cross-reactive with different strains of the virus have been described with vesicular stomatitis virus, influenza virus, alphaviruses, murine leukemia viruses, reovirus, etc. (16, 35, 42). In general, the cross-reactive CTL predominate as noted in the influenza virus, vesicular stomatitis virus, and alphavirus systems. Results with these viruses have shown that serologically distinct viruses often induce cross-reactive CTL, suggesting that virus-specific CTL react with different determinants on viral proteins than do virus-specific antibodies. However, the specific viral protein(s) recognized by CTL is known only in a limited number of virus systems, and except for the reovirus system, there is no information regarding which epitopes on viral proteins are seen by CTL (4, 7, 16–20, 35, 36, 38, 42). At the moment, the LCMV determinant(s) recognized by CTL is unknown. Future studies with LCMV reassortants, transfection of cells with cloned LCMV genes, and site-directed in vitro mutagenesis of cloned LCMV genes should identify the LCMV protein(s) and its epitopes recognized by CTL.

It is well established that antibody response is under genetic control (23, 27). These immune response genes map to the I-region (class II gene products) of the MHC and regulate both the magnitude and the specificity of the antibody response to a variety of antigens (2, 3, 26). Genetic regulation of CTL response was first described for trinitrophenyl-specific and H-Y antigen-specific CTL (21, 34). Several studies have shown that CTL generated during acute viral infections of mice are also controlled by immune response genes (41). Unlike the classical immune response genes, these immune response phenomena map to the *H-2K* or *H-2D* region (class I gene products) of the MHC. Genetic control of CTL response has been demonstrated for influenza virus, vaccinia virus, vesicular stomatitis virus, Sendai virus, LCMV, and alphaviruses (5, 13, 22, 29, 30, 33, 39). These studies have shown that the *H-2* region controls the magnitude of the CTL response and that low responsiveness or nonresponsiveness is associated with the *H-2K* or *D* allele and, in some instances, with the entire *H-2* genotype. For example, *H-2*^{k} mice are unable to generate CTL to vesicular stomatitis virus (33). However, the effect of the *H-2* region on the fine specificity of virus-specific CTL has not been extensively examined except for a recent study showing that *H-2*^{d}- and *H-2*^{b}-restricted influenza virus-specific CTL clones exhibit different cross-reactivity patterns (37). These results complement our findings that the cross-reactivity pattern of LCMV-specific CTL is determined by the gene products of the *H-2* region. It is significant that the different cross-reactivity patterns of *H-2*^{b}- and *H-2*^{d}-restricted LCMV-specific CTL can be observed at the population level, indicating that the degree of cross-protective immunity generated during infection will vary depending on the MHC haplotype. Thus, this type of genetic control over virus-specific CTL may, in part, explain the failure of vaccines to provide immunity against viral infections to all members within a heterogeneous population.

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