

SELECTION OF GENETIC VARIANTS OF LYMPHOCYTIC  
CHORIOMENINGITIS VIRUS IN SPLEENS OF  
PERSISTENTLY INFECTED MICE  
Role in Suppression of Cytotoxic T Lymphocyte Response  
and Viral Persistence

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The mechanisms by which viruses evade or suppress the immune response and persist in the host is one of the most intriguing problems in virology. A variety of viruses, such as herpes simplex, cytomegalovirus, Epstein-Barr virus, rubella, measles, hepatitis B, etc., are known to cause persistent infections in humans (1). Persistent viral infections are medically significant due to their association with a variety of diseases (1). In recent years, it has become evident that a number of chronic human diseases such as subacute sclerosing panencephalitis, progressive multifocal leukoencephalopathy, and progressive rubella panencephalitis are caused by persistent viral infections (2, 3). A viral etiology is also suspected in several other chronic human illnesses (2, 3). In addition, the persistence of hepatitis B virus in an estimated 200 million persons constitutes a major public health problem of worldwide concern (4). Thus, there is a need to understand the host and viral factors involved in the establishment and maintenance of persistent infections.

Persistent infection of the mouse with lymphocytic choriomeningitis virus (LCMV)<sup>1</sup> is an excellent experimental model for studying the interaction between the virus and the host's immune system, and in defining the conditions that lead to viral clearance or persistence (5, 6). LCMV is maintained in nature as a persistent infection (carrier state) in mice, as evident in many wild mice of the *Mus musculus* strain. The life-long carrier state is readily established in laboratory mice by injecting them at birth with LCMV. Such carrier mice make antibody responses against the virus, but contain minimal amounts of or no detectable

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<sup>1</sup> *Abbreviations used in this paper:* CTL, cytotoxic T lymphocytes; EIA, equine infectious anemia virus; ELISA, enzyme-linked immunosorbent assay; IC, infectious centers; LCMV, lymphocytic choriomeningitis virus; MOI, multiplicity of infection; PBS, phosphate-buffered saline; PFU, plaque-forming units.

LCMV-specific cytotoxic T lymphocytes (CTL) (6–10). This classic model of viral persistence has been extensively studied during the past 50 years, but a major unresolved question is the mechanism of suppression of CTL responses and its role in persistence (5, 6, 11).

In this paper, we describe our studies on the mechanism of LCMV persistence and suppression of CTL responses in BALB/c WEHI mice infected with LCMV Armstrong strain. We found that spleen cells from carrier mice actively suppress the expected LCMV-specific CTL response of spleen cells from normal adult mice, and that this suppression is due to the presence of genetic variants of LCMV in spleens of carrier mice. We propose that these LCMV variants, generated during infection *in vivo*, play a crucial role in the maintenance of the carrier state in mice.

### Materials and Methods

*Mice.* BALB/c WEHI (H-2<sup>d</sup>) mice were obtained from the breeding colony at Scripps Clinic and Research Foundation.

*Virus.* The origin of the LCMV Armstrong CA1371 strain used in this study has been described (12). The virus was triple plaque-purified on Vero cells and grown in BHK-21 cells. Virus stocks at the passage 1 or passage 2 level were used in all experiments. LCMV clones 1, 3, 13, and 14 were isolated from the spleens of 2-mo-old BALB/c WEHI carrier mice infected at birth with LCMV Armstrong CA1371. Spleens from three carrier mice were pooled, homogenized, and centrifuged at 1,200 rpm for 10 min to remove the cellular debris. The clarified homogenate was titrated on Vero cell monolayers and isolated plaques were picked from the plates. These clones were subjected to two additional cycles of plaque to plaque purification and then grown in BHK-21 cells. All four spleen isolates, clones 1, 3, 13, and 14, were authenticated as LCMV by their positive reactivity with monoclonal antibodies specific for the nucleoprotein and glycoprotein of LCMV Armstrong (13). Vaccinia virus (IHD-J strain) used in this study was a gift of Dr. Sam Dales, University of Western Ontario, London, Ontario, Canada.

*Adoptive Transfer Experiments.* 6–10-wk-old normal or carrier BALB/c WEHI mice were used as donors of spleen cells. After sacrifice, their spleens were harvested and single-cell suspensions were prepared in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, antibiotics, and L-glutamine (complete RPMI). Erythrocytes were removed by treatment with 0.83% NH<sub>4</sub>Cl solution. One pool was made of spleen cells from normal mice and another of spleen cells from carrier mice.  $5 \times 10^7$  viable spleen cells were transferred intravenously into 2–4-mo-old normal or carrier BALB/c WEHI mice irradiated (600 rad) 1 d previously. Mice reconstituted with both carrier and normal spleen cells received  $5 \times 10^7$  cells from carrier mice and  $5 \times 10^7$  cells from normal mice. At the time of the cell transfer all recipient mice were infected intravenously with the indicated dose and strain of LCMV. In some experiments mixtures of spleen and lymph node cells (ratio, 3:1) were used to reconstitute the irradiated mice.

*Cytotoxicity Assay.* Single-cell suspensions of spleens, free of erythrocytes, were prepared in complete RPMI medium and tested for cytotoxicity on uninfected and LCMV-infected BALB Cl-7 (H-2<sup>d</sup>) fibroblasts. BALB Cl-7 cells were infected with the indicated LCMV strain at a multiplicity of infection (MOI) of 0.5 plaque-forming units (PFU) per cell and used 18–24 h later for the assay. The target cells were labeled with <sup>51</sup>Cr and the cytotoxicity assay was performed as previously described (14, 15). The test duration was 6–8 h. The percent specific <sup>51</sup>Cr release was calculated by the formula: [(Sample release – spontaneous release [cpm]) / (maximum release – spontaneous release [cpm])] × 100. Vaccinia virus CTL assays were done in the same manner except that B.10.D2 (H-2<sup>d</sup>) fibroblasts were used as targets. The B.10.D2 cells were infected with vaccinia virus (IHD-J strain) at an MOI of 10 PFU/cell and used 2 h later for the <sup>51</sup>Cr-release assay. Test duration was 6 h. All samples were assayed in triplicate and standard error was <2%.

*Virus Titrations.* Infectious LCMV was quantitated by plaque assay on Vero cell monolayers.  $7.5 \times 10^5$  Vero cells were plated in 35-mm wells in 6-well dishes (Costar, Cambridge, MA). The plates were incubated at 37°C and used the following day for the assay when the cell monolayers were confluent. The medium was removed and the samples to be titrated were added to the cells (0.2 ml vol). After adsorption for 60 min at 37°C, the cells were overlaid with 3 ml of 0.5% Seakem agarose (FMC Corporation, Rockland, ME) in Medium 199 (Gibco Laboratories, Grand Island, NY) supplemented with 5% heat-inactivated fetal calf serum, antibiotics, and L-glutamine. The plates were incubated for 5 d at 37°C and then overlaid with 2.0 ml of 0.5% agarose in Medium 199 containing 0.01% neutral red (Gibco Laboratories). Plaques were scored the following day.

*Antibody Titrations.* LCMV-specific antibody was measured by a solid phase enzyme-linked immunosorbent assay (ELISA) using purified virus as the antigen. LCMV Armstrong strain was grown in BHK-21 cells and purified on Renografin gradients as previously described (12). The purified virus was diluted in phosphate-buffered saline (PBS), sonicated to disrupt clumps, and the protein concentration was determined by Lowry's method (16) using bovine serum albumin as the standard. Microtiter plates (Linbro Titertek; Flow Laboratories, McLean, VA) were coated with 200 ng/well of viral antigen in a 0.1 ml vol. After overnight incubation at room temperature, free binding sites of the polystyrene wells were blocked with 0.15 ml/well of PBS supplemented with 10% (vol/vol) heat-inactivated fetal calf serum, 0.2% (vol/vol) Tween 20, and 0.5 mM merthiolate. After 1 h or more incubation at room temperature, antigen-coated plates were used for antibody-binding assays. Viral antigen-coated plates prepared in this manner showed a high level of binding to LCMV-specific monoclonal antibodies directed against the LCMV nucleocapsid protein and the two glycoproteins, GP-1 and GP-2 (13). Thus, this method allows us to measure antibodies directed against the three major structural proteins of LCMV. To measure LCMV-specific antibody in the serum samples, serial threefold dilutions of the samples were incubated in antigen-coated plates for 75–90 min at room temperature. The plates were washed three times with PBS containing 0.05% (vol/vol) Tween 20, and horseradish peroxidase-labeled, affinity-purified antibodies to mouse IgG and IgM (Boehringer Mannheim Biochemicals, Indianapolis, IN) were added. After 75–90 min incubation, the plates were washed as above and 0.1 ml of the substrate solution, consisting of 3 mM hydrogen peroxide and 2.2 mM *o*-phenylenediamine in 0.1 M sodium citrate buffer, pH 5.0, was added to each well. The enzyme reaction was terminated after 30 min by adding 0.1 ml of 1 N HCl into each well. Optical densities were read (OD<sub>492</sub>) with a Titertek Multiskan photometer (Eflab, Helsinki, Finland). In all experiments, plates coated with purified measles virus (Edmonston strain) (200 ng/well) were included as controls. The LCMV-specific antibody titer was determined by subtracting the binding (OD<sub>492</sub>) to measles virus-coated plates from the binding (OD<sub>492</sub>) to LCMV-coated plates. The LCMV-specific antibody titer is expressed as the reciprocal of the highest dilution showing an OD<sub>492</sub> reading >2 standard deviations from the OD<sub>492</sub> reading of serum samples from mock-infected mice (average of at least six control mice).

## Results

*LCMV-specific CTL and Antibody Responses During Acute and Persistent LCMV Infection.* Infection of adult BALB/c WEHI mice with LCMV Armstrong induced a vigorous cellular and humoral response against the virus. Table I shows the LCMV-specific CTL and antibody response of six adult mice infected with  $2 \times 10^5$  PFU i.v. of LCMV Armstrong 8 d previously. All six mice had high levels of CTL and antibody. Such acutely infected mice cleared the virus within 8–10 d postinfection (data not shown). When BALB/c WEHI mice are infected at birth with LCMV Armstrong (by any route of injection), they become carriers with life-long viremia and contain high levels of infectious LCMV in all major organs. We have checked for LCMV-specific CTL in the spleens and lymph

TABLE I  
*LCMV-specific CTL and Antibody Responses in Acutely and Persistently Infected BALB/c WEHI Mice*

Status of mice	Mouse No.	CTL response*						Antibody response <sup>‡</sup> LCMV-specific anti-body ELISA titer (log 2)
		Percent specific <sup>51</sup> Cr release from BALB Cl-7 (H-2 <sup>d</sup> ) targets						
		Uninfected			LCMV Armstrong infected			
		E/T ratio			E/T ratio			
		5:1	25:1	50:1	5:1	25:1	50:1	
Mock infected <sup>§</sup>	1	0	0	3	0	0	1	<4.7
	2	0	1	8	1	3	3	<4.7
	3	2	4	3	3	5	6	<4.7
	4	3	2	5	0	4	7	<4.7
	5	0	5	6	2	4	9	<4.7
	6	0	5	9	3	3	7	<4.7
Acutely infected <sup>¶</sup>	1	1	3	5	22	37	79	15.3
	2	1	1	3	12	39	63	15.9
	3	2	1	5	34	67	76	15.9
	4	0	2	1	28	45	72	15.8
	5	0	4	4	21	44	78	15.7
	6	2	3	7	19	51	62	16.3
Persistently infected <sup>  </sup> (carrier mice)	1	0	0	0	0	0	2	12.0
	2	0	4	5	2	2	6	<4.7
	3	2	3	3	2	2	4	<4.7
	4	4	2	3	4	5	7	<4.7
	5	5	2	4	2	2	5	9.8
	6	0	1	5	0	1	3	7.5

\* CTL activity in the spleen was determined by a <sup>51</sup>Cr release assay. Test duration was 6–8 h. Spontaneous release was <20%. All samples were assayed in triplicate and standard error was <2%. E/T, effector/target.

<sup>‡</sup> LCMV-specific antibody in serum was determined by an ELISA using purified virus as antigen.

<sup>§</sup> 8-wk-old mice injected intravenously with 0.2 ml of medium. LCMV-specific CTL and antibody were checked 7 d later.

<sup>¶</sup> 8-wk-old mice injected intravenously with 2 × 10<sup>5</sup> PFU of LCMV Armstrong. LCMV-specific CTL and antibody were checked 7 d postinfection.

<sup>||</sup> Mice injected intracerebrally with 10<sup>4</sup> PFU of LCMV Armstrong at birth (<24 h old). LCMV-specific CTL and antibody were checked when mice were 8-wk-old.

nodes of 1–3-mo-old carrier mice in many different experiments and have not detected any CTL activity. The level of killing exhibited by spleen cells from six 2-mo-old carrier mice on LCMV-infected and uninfected syngeneic targets is shown in Table I. The killing is minimal and not significantly higher than that by spleen cells from mock-infected BALB/c WEHI mice (Table I). In contrast to the absence of detectable CTL activity, LCMV-specific antibody was present in the sera of BALB/c WEHI carrier mice. However, the amount of antibody was low and detectable in only three out of six mice (Table I). It should be

pointed out that the ELISA test we used measures free antibody and that LCMV-specific antibody present in immune complexes in carrier mice is not detected by this method.

*Effect of Normal and Carrier Environment on the Generation of LCMV-specific CTL and Antibody Responses.* To further investigate the mechanism of low responsiveness of BALB/c WEHI carrier mice to LCMV, we adoptively transferred spleen cells from carriers into irradiated normal recipients (C → N) and spleen cells from normal adult mice into irradiated carrier recipients (N → C). All recipient mice were injected intravenously with LCMV Armstrong at the time of cell transfer, and LCMV-specific antibody and CTL responses were checked 8 d postinfection. As the results in Table II show, normal irradiated mice reconstituted with normal spleen cells (N → N) made potent LCMV-specific CTL and antibody responses. In contrast, C → N mice made no detectable CTL response and a low antibody response. Since the irradiated normal recipient mice provide a source of antigen-presenting cells, this result shows that the low responsiveness of carrier spleen cells to LCMV is not simply due to a defect in antigen-presenting cells. When normal spleen cells were transferred into irradiated carrier recipients (N → C), there was no detectable CTL activity. This was in striking contrast to the high CTL activity seen in N → N mice. However, N → C mice made an LCMV-specific antibody response about equivalent to N → N mice. These results show that the carrier environment suppressed the LCMV-specific CTL response of spleen cells from normal adult mice but had negligible effect on LCMV-specific antibody response.

Spleen cells from LCMV carrier mice do not have a generalized defect in generating antiviral CTL responses, as shown in Table III. The magnitude of the vaccinia-specific CTL response upon challenge with vaccinia virus is about equal in N → N and C → N mice.

*Spleen Cells from Carrier Mice Actively Suppress LCMV-specific CTL Response of Spleen Cells from Normal Adult Mice But Have No Effect on LCMV-specific Antibody Response.* Because the carrier environment actively suppressed LCMV-specific CTL responses (Table II), we next examined whether carrier spleen cells could transfer the suppression. Irradiated normal mice were reconstituted with either (a)  $5 \times 10^7$  spleen cells from normal mice (N → N), (b)  $5 \times 10^7$  spleen cells from carrier mice (C → N), or (c) equal mixtures ( $5 \times 10^7 + 5 \times 10^7$ ) of spleen cells from carrier and normal mice (C + N → N). One group of irradiated mice was not given spleen cells (None → N). In some experiments, mixtures of spleen and lymph node cells (ratio, 3:1) were used to reconstitute the irradiated mice. The results of these experiments were identical to those using spleen cells alone. All irradiated recipient mice were challenged with LCMV Armstrong at the time of the cell transfer, and, 1, 3, 6, 8, 10, and 15 d later, were checked for LCMV-specific CTL, LCMV-specific antibody, and infectious LCMV in spleens (Fig. 1). As expected, None → N mice did not generate any detectable immune response against LCMV and contained high levels of virus in their spleens. N → N mice made strong LCMV-specific CTL and antibody responses and cleared the virus infection by day 10. Their CTL and antibody responses peaked at day 8, but the CTL activity disappeared by day 15, while antibody remained at high levels. C → N mice made no detectable CTL response, a minimal antibody response

TABLE II

*Inability of Spleen Cells from Carrier Mice to Generate LCMV-specific CTL Responses in Normal Recipients, and Suppression of CTL Response of Spleen Cells from Normal Adult Mice in Carrier Recipients*

Source of spleen cells transferred <sup>‡</sup>	Recipient (600 rad)	LCMV-specific CTL in spleen (8 d post-infection)				LCMV-specific antibody in serum (8 d postinfection) ELISA titer (log 2)
		Percent specific <sup>51</sup> Cr release from BALB Cl-7 (H-2 <sup>d</sup> ) targets*				
		Uninfected		LCMV Armstrong infected		
		E/T ratio		E/T ratio		
		25:1	50:1	25:1	50:1	
None	Normal	0	NT <sup>§</sup>	1	NT	<4.7
None	Normal	0	NT	0	NT	<4.7
None	Normal	0	NT	0	NT	<4.7
None	Normal	0	NT	0	NT	<4.7
Normal	Normal	3	1	57	64	15.8
Normal	Normal	0	2	58	62	15.1
Normal	Normal	0	3	49	54	15.3
Normal	Normal	4	3	38	59	14.6
Carrier	Normal	2	0	1	0	4.7
Carrier	Normal	0	0	2	0	<4.7
Carrier	Normal	3	0	1	2	5.6
Carrier	Normal	0	0	0	1	<4.7
None	Carrier	0	NT	0	NT	<4.7
None	Carrier	2	NT	0	NT	6.8
None	Carrier	0	NT	2	NT	<4.7
None	Carrier	1	NT	1	NT	7.1
Normal	Carrier	0	5	1	0	14.3
Normal	Carrier	0	0	1	3	14.5
Normal	Carrier	3	2	0	1	14.9
Normal	Carrier	0	0	0	2	14.6

\* Test duration was 6–8 h. Spontaneous release was <20%. All samples were assayed in triplicate and standard error was <2%.

<sup>‡</sup>  $5 \times 10^7$  spleen cells from 6–10-wk-old BALB/c WEHI mice were transferred intravenously into 2–4-mo-old BALB/c WEHI mice irradiated (600 rad) 1 d previously. At the time of the cell transfer all recipient mice were infected intravenously with  $2 \times 10^5$  PFU of LCMV Armstrong. LCMV-specific antibody and CTL were checked 8 d postinfection.

<sup>§</sup> Not tested.

~1,000-fold lower than N → N mice), and were unable to clear the infection. C + N → N mice made vigorous LCMV-specific antibody responses but poor CTL responses, and contained high levels of virus in the spleen until 15 d postinfection. Since CTL activity was measured by a <sup>51</sup>Cr-release assay, it could be argued that LCMV-infected cells in the spleens of N + C → N mice acted as “cold” competitors in the cytotoxicity assay, thus exerting a pseudosuppressive effect. This was tested by mixing spleen cells from C + N → N mice with spleen cells from N → N mice

TABLE III  
*Spleen Cells from LCMV Carrier Mice Can Generate Vaccinia Virus-specific CTL Response*

Source of spleen cells transferred*	Experiment 1		Experiment 2	
	Percent specific <sup>51</sup> Cr release from B.10.D2 (H-2 <sup>d</sup> ) targets <sup>‡</sup>		Percent specific <sup>51</sup> Cr release from B.10.D2 (H-2 <sup>d</sup> ) targets <sup>‡</sup>	
	Uninfected	Vaccinia infected	Uninfected	Vaccinia infected
None	0	9	0	14
	0	7	0	9
Normal	2	48	2	61
	2	52	3	69
	0	43	1	61
	0	50		
Carrier	0	52	2	46
	0	22	6	64
	0	43	2	60
	0	51		
	0	28		

\*  $5 \times 10^7$  spleen cells from 6–10-wk-old BALB/c WEHI mice were transferred intravenously into 2–4-mo-old normal BALB/c WEHI mice irradiated (600 rad) 1 d previously. At the same time, all recipient mice were infected intravenously with  $10^6$  PFU of vaccinia virus (1HD-J strain).

<sup>‡</sup> Mice were sacrificed 7 d postinfection and CTL activity was checked on vaccinia virus-infected and uninfected B.10.D2 (H-2<sup>d</sup>) targets in a 6 h <sup>51</sup>Cr release assay. Spontaneous release was 15–30%. The data shown is percent specific killing at an effector/target ratio of 50:1.

in a 1:1 ratio during the <sup>51</sup>Cr-release assay; however, no inhibition occurred (data not shown). Thus, the low level of killing by spleen cells from C + N → N mice does represent fewer CTL. Our results show that spleen cells from carrier mice actively suppress the LCMV-specific CTL response of normal adult spleen cells. The suppression is specific for the CTL response and has no effect on LCMV-specific antibody response. The kinetics and magnitude of the LCMV-specific antibody response were similar in N → N and C + N → N mice. The LCMV-specific antibody was predominantly (>80%) of the IgG class in both N → N and C + N → N mice (data not shown). It should be noted that N → N mice that made both antibody and CTL responses against LCMV cleared virus from their spleens by day 10, whereas C + N → N mice that made potent LCMV-specific antibody responses but poor or undetectable CTL responses contained high levels of infectious LCMV in their spleens at day 15.

*Suppression of LCMV-specific CTL Response Is Associated with the Establishment of Persistent LCMV Infection.* Groups of N → N, C → N, and C + N → N mice were examined for viremia by sampling blood on days 28, 41, 68, and 99 postinfection and determining LCMV titers by plaque assay on Vero cell monolayers. N → N mice had no detectable LCMV, whereas C → N and C + N → N

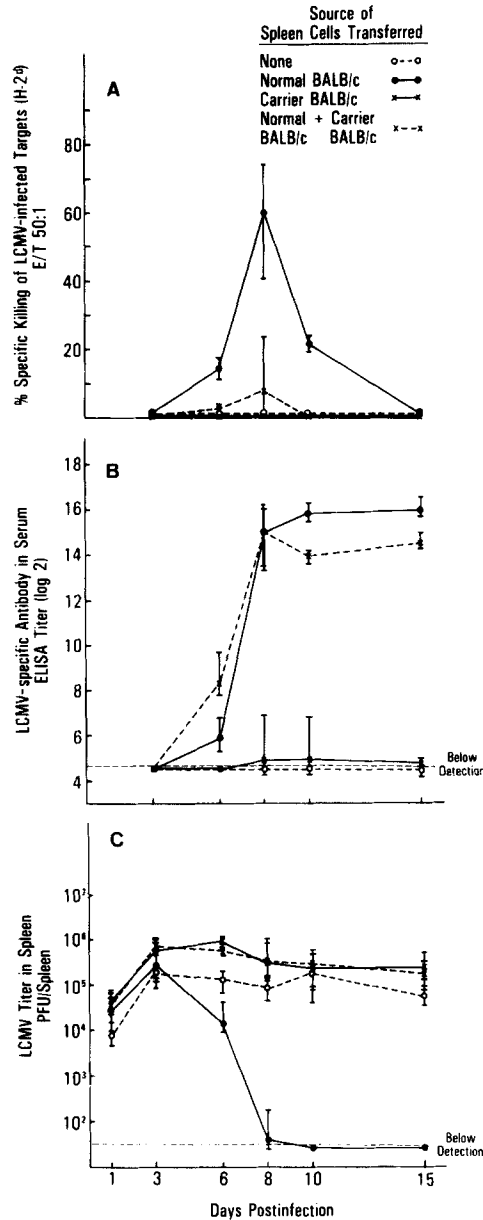


FIGURE 1. Effect of carrier spleen cells on the generation of LCMV-specific CTL and antibody responses. The suppression of CTL response is associated with the inability to clear virus infection.  $5 \times 10^7$  spleen cells from the indicated source were transferred intravenously into 2–4-mo-old normal BALB/c mice irradiated (600 rad) 1 d previously. Mice reconstituted with both carrier and normal spleen cells received  $5 \times 10^7$  cells from each source. At the time of the cell transfer all recipient mice were infected intravenously with  $2 \times 10^5$  PFU of wild-type LCMV Armstrong. At various times postinfection mice were checked for (A) LCMV-specific CTL, (B) LCMV-specific antibody, and (C) LCMV titer in spleen. CTL activity in the spleen was determined by a 6–8 h  $^{51}\text{Cr}$ -release assay. LCMV-specific antibody was measured by an ELISA test using purified virus as antigen. The titer of infectious LCMV was determined by plaque assay on Vero cell monolayers. Each point represents the mean of at least three mice. The day 8 point contains >20 mice. The bars indicate the range.



TABLE IV  
*Irradiated Mice Reconstituted with Mixtures of Spleen Cells from Normal and Carrier Mice Fail to Clear the Virus Infection and Become LCMV Carriers*

Source of spleen cells transferred*	Normal recipient (600 rad) No.	LCMV titer in serum <sup>‡</sup> (log <sub>10</sub> PFU/ml)				LCMV titer at 99 d postinfection (log <sub>10</sub> PFU/organ) <sup>§</sup>				
		Days postinfection				Lymph node (inguinal)	Thymus	Spleen	Kidney	Brain
		28	41	68	99					
Normal	1	<1.3	<1.3	<1.3	<1.3	<1.6	<1.6	<1.6	<1.6	<1.6
	2	<1.3	<1.3	<1.3	<1.3	<1.6	<1.6	<1.6	<1.6	<1.6
	3	<1.3	<1.3	<1.3	<1.3	<1.6	<1.6	<1.6	<1.6	<1.6
Carrier	1	3.5	3.6	5.0	5.1	6.1	5.5	5.9	6.7	5.9
	2	3.5	3.8	5.0	5.5	4.8	5.1	5.0	6.3	5.7
	3	3.4	3.9	5.1	4.9	5.5	6.1	5.2	6.9	4.9
Normal plus carrier	1	3.6	3.8	4.7	4.7	5.7	6.1	5.0	6.8	5.5
	2	3.8	3.5	4.9	4.9	5.6	5.5	5.8	6.6	6.1
	3	3.9	3.8	4.5	5.0	5.2	5.0	4.9	6.4	5.6
	4	3.3	3.3	4.4	4.6	5.9	5.8	5.7	6.7	5.7

\*  $5 \times 10^7$  spleen cells from 6–10-wk-old BALB/c WEHI mice were transferred intravenously into 2–4-mo-old normal BALB/c WEHI mice irradiated (600 rad) 1 d previously. Mice reconstituted with both carrier and normal spleen cells received  $5 \times 10^7$  cells from carrier mice and  $5 \times 10^7$  cells from normal mice. At the time of the cell transfer all recipient mice were infected intravenously with  $2 \times 10^5$  PFU of LCMV Armstrong.

<sup>‡</sup> Mice were eye-bled and the virus titer in the serum was determined by titration on Vero cell monolayers.

<sup>§</sup> Mice were sacrificed and the various organs harvested, homogenized, and titrated on Vero cell monolayers.

mice contained high levels of infectious LCMV (Table IV). The mice were sacrificed 99 d after infection, at which time we found high levels of LCMV in the spleens, lymph nodes, thymuses, kidneys, and brains of all C → N and C + N → N mice tested (Table IV). Thus, these C → N and C + N → N mice became long-term carriers. Most interestingly, C + N → N mice failed to clear the virus infection despite making a strong LCMV-specific antibody response. Taken together, these results demonstrate that the establishment of persistent LCMV infection is associated with the suppression of LCMV-specific CTL response.

We next tested whether the transfer of spleen or lymph node cells containing LCMV-specific CTL would result in virus clearance and prevent establishment of the carrier state. Spleen or lymph node cells from normal adult mice infected 8 d previously with LCMV Armstrong were used as a source of LCMV-specific CTL.  $5 \times 10^7$  day 8 spleen or lymph node cells were mixed with  $5 \times 10^7$  carrier spleen cells and transferred intravenously into irradiated normal mice. At the time of the cell transfer all recipient mice were infected with LCMV Armstrong. Mice were sacrificed at days 3 and 7 postinfection and the virus titer in various organs and sera was determined by plaque assay on Vero cells (Table V). As before (Fig. 1 and Table IV), mice reconstituted with carrier spleen cells alone contained high levels of virus. In contrast, mice reconstituted with mixtures of

TABLE V  
*Transfer of Spleen or Lymph Node Cells Containing LCMV-specific CTL Results in Virus Clearance and Prevents Establishment of the Carrier State*

Source of cells transferred*	LCMV titer (log <sub>10</sub> PFU/organ or ml <sup>†</sup> )									
	Day 3 postinfection					Day 7 postinfection				
	Spleen	Axillary lymph node	Inguinal lymph node	Thymus	Serum	Spleen	Axillary lymph node	Inguinal lymph node	Thymus	Serum
Carrier spleen	6.2	6.0	5.4	4.9	<1.6	6.3	5.8	5.7	5.8	4.3
	6.2	6.1	6.0	4.9	<1.6	6.3	5.6	5.4	5.8	4.6
	6.0	5.2	5.0	4.6	<1.6	6.1	5.2	5.3	5.7	4.3
Carrier spleen + day 8 spleen <sup>‡</sup>	<1.6	5.4	5.0	3.3	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
	<1.6	5.3	5.0	4.1	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
	<1.6	5.0	5.2	3.2	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
Carrier spleen + day 8 axillary LN <sup>‡</sup>	5.8	5.1	5.2	4.0	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
	5.7	5.3	4.5	3.9	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
	4.3	4.4	4.8	4.0	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
Carrier spleen + day 8 inguinal LN <sup>‡</sup>	5.3	5.2	4.4	4.0	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
	5.5	5.5	5.3	4.2	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
	5.4	5.1	5.0	4.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6

\*  $5 \times 10^7$  cells from 6–10-wk-old BALB/c WEHI mice were transferred intravenously into 2–4-mo-old normal BALB/c WEHI mice irradiated (600 rad) 1 d previously. Mice reconstituted with both carrier spleen cells and day 8 spleen (or day 8 LN<sup>‡</sup>) cells received  $5 \times 10^7$  cells from each source. At the time of the cell transfer all recipient mice were infected intravenously with  $2 \times 10^5$  PFU of LCMV Armstrong.

<sup>†</sup> Mice were sacrificed and the virus titer in the various organs and serum determined by plaque assay on Vero cell monolayers.

<sup>‡</sup> Spleen and lymph node (LN) cells from 6–10-wk-old normal BALB/c WEHI mice infected intravenously 8 d previously with  $2 \times 10^5$  PFU of LCMV Armstrong.

carrier spleen cells and day 8 spleen or lymph node cells cleared the virus infection within 7 d. These mice contained no detectable infectious LCMV in the serum or any of the organs tested. It should be noted that day 8 lymph node cells did not reduce the virus titer in any organ at 3 d postinfection, whereas day 8 spleen cells had effectively cleared the infection from the spleen by day 3 postinfection although high levels of LCMV were present in the lymph nodes and the thymus. This may represent preferential homing of day 8 spleen cells into the spleen of recipient mice.

*Suppression of LCMV-specific CTL Response by Carrier Spleen Cells Is Not Mediated by a Suppressor Cell.* Carrier spleen cells subjected to x irradiation (3,000 rad), anti-theta plus complement treatment, or sonication were tested for their ability to suppress LCMV-specific CTL responses. As Table VI shows, none of these treatments abrogated suppression, indicating that inhibition of LCMV-specific CTL response by carrier spleen cells does not require intact cells and is unlikely to be due to suppressor cells. As in the earlier experiments, LCMV-specific antibody responses were not affected, and CTL suppression was associated with an inability to clear the virus infection.

*Suppression of LCMV-specific CTL Response Is Due to the Presence of Genetic Variants of LCMV in Carrier Spleen Cells.* Approximately 1% of spleen cells from BALB/c WEHI carrier mice score as LCMV infectious centers (IC). In these transfer experiments carrier spleen cells contained 0.28–2.4% LCMV IC. Since  $5 \times 10^7$  carrier spleen cells were transferred into each recipient, the number of LCMV IC transferred ranged from  $1.4 \times 10^5$  to  $1.2 \times 10^6$ . When the carrier

TABLE VI  
*Suppression of LCMV-specific CTL Response by Carrier Spleen Cells Is Not Abrogated by x Irradiation, Anti-theta Treatment, or Sonication of Carrier Spleen Cells*

Source of spleen cells transferred*	Treatment of carrier spleen cells <sup>‡</sup>	LCMV-specific CTL in spleen <sup>§</sup>		LCMV-specific antibody in serum <sup>§</sup>	LCMV titer in spleen <sup>§</sup>
		Percent specific <sup>51</sup> Cr release from BALB C1-7 (H-2 <sup>d</sup> ) targets (E/T, 50:1)			
		Uninfected	LCMV Arm-strong infected	ELISA titer (log 2)	Log <sub>10</sub> PFU/spleen
Normal (N)		8	68	14.8	<1.6
		7	64	16.0	<1.6
		14	65	13.3	<1.6
Carrier (C)	None	1	0	<4.9	4.3
		3	1	<4.9	4.5
		2	2	<4.9	5.3
N + C	None	2	11	16.3	5.5
		0	8	15.8	5.3
		1	6	14.7	5.5
N + C	Anti-theta + C'	0	16	15.9	5.6
		6	14	16.4	5.2
		0	13	16.0	5.1
N + C	3,000 rad	0	0	13.7	4.1
		0	0	14.0	3.7
N + C	Sonication	4	13	15.5	5.2
		6	15	16.4	5.3
		0	8	14.7	4.7

\*  $5 \times 10^7$  spleen cells from 6–10-wk-old BALB/c WEHI mice were transferred intravenously into 2–4-mo-old normal BALB/c WEHI mice irradiated (600 rad) 1 d previously. At the time of the cell transfer all recipient mice were infected intravenously with  $2 \times 10^5$  PFU of LCMV Armstrong.

<sup>‡</sup>  $5 \times 10^7$  spleen cells from carrier mice were treated as indicated and then mixed with  $5 \times 10^7$  spleen cells from normal mice and transferred intravenously into irradiated recipients.

<sup>§</sup> LCMV-specific CTL, antibody, and virus titer were checked 8 d postinfection. E/T, effector/target.

spleen cells were sonicated, titration of the sonicated lysate revealed that  $4 \times 10^5$  PFU of LCMV were transferred into each recipient. We have found that injection of up to  $10^7$  PFU of LCMV Armstrong induces a CTL response, and that the infection is cleared within 8–10 d (data not shown). This suggested that, if suppression of CTL response was due to the carried-over virus, the LCMV present in spleens of carrier mice may be a genetic variant or in a form that induces suppression. To check this possibility, LCMV was isolated from the spleens of 2-mo-old carrier mice infected at birth with LCMV Armstrong. Spleens from three carrier mice were pooled, homogenized, and titrated on Vero cell

monolayers. Well-isolated plaques were picked and subjected to two additional cycles of plaque to plaque purification and then grown up in BHK-21 cells. Four such triple-cloned LCMV spleen isolates, clones 1, 3, 13, and 14, were tested for their ability to induce LCMV-specific CTL and antibody responses (Table VII). Irradiated normal mice were reconstituted with  $5 \times 10^7$  spleen cells from normal mice and challenged with either LCMV Armstrong or one of the LCMV spleen isolates. Mice infected with  $2 \times 10^5$  or  $2 \times 10^6$  PFU of LCMV Armstrong generated good CTL and antibody responses and cleared the virus from their spleens by day 8 postinfection. One of four mice had residual amounts ( $2.3 \log_{10}$

TABLE VII  
*Suppression of LCMV-specific CTL Response by Carrier Spleen Cells Is Due to the Presence of Genetic Variants of LCMV in Carrier Mice*

Source of spleen cells transferred*	Challenge virus	Dose (PFU)	LCMV-specific CTL in spleen <sup>‡</sup>			LCMV-specific antibody in serum <sup>†</sup>	LCMV titer in spleen <sup>‡</sup>
			Percent specific <sup>51</sup> Cr release from BALB Cl-7 (H-2 <sup>d</sup> ) targets (E/T, 50:1)				
			Uninfected	LCMV Armstrong infected	LCMV clone 13 infected	ELISA titer (log 2)	Log <sub>10</sub> PFU/spleen
Normal	LCMV Armstrong	$2 \times 10^5$	0	62	52	12.5	<1.6
		$2 \times 10^5$	0	41	38	12.9	<1.6
		$2 \times 10^6$	0	53	40	14.0	2.3
		$2 \times 10^6$	2	59	47	13.6	<1.6
Normal	LCMV clone 1	$2 \times 10^5$	0	17	19	11.3	3.8
		$2 \times 10^5$	11	18	18	14.1	4.1
		$2 \times 10^6$	0	16	24	12.7	4.3
		$2 \times 10^6$	0	24	14	11.2	4.7
Normal	LCMV clone 3	$2 \times 10^5$	0	16	22	11.6	4.0
		$2 \times 10^5$	0	7	0	10.9	4.8
		$2 \times 10^6$	0	16	15	12.7	4.9
		$2 \times 10^6$	0	8	0	15.0	5.0
Normal	LCMV clone 13	$2 \times 10^5$	0	7	2	11.6	5.2
		$2 \times 10^5$	0	6	0	12.4	5.1
		$2 \times 10^6$	0	14	18	12.8	5.0
		$2 \times 10^6$	0	19	22	11.9	5.1
Normal	LCMV clone 14	$2 \times 10^5$	0	12	19	12.3	4.4
		$2 \times 10^5$	0	18	19	12.7	4.1
		$2 \times 10^6$	0	7	7	11.8	3.9
		$2 \times 10^6$	0	6	7	12.7	3.9

\*  $5 \times 10^7$  spleen cells from adult BALB/c WEHI mice were transferred intravenously into normal adult BALB/c WEHI mice irradiated (600 rad) 1 d previously. At the same time recipient mice were infected intravenously with the indicated virus. LCMV clones 1, 3, 13, and 14 were isolated from the spleens of 2-mo-old carrier mice infected at birth with LCMV Armstrong.

<sup>‡</sup> LCMV-specific CTL, antibody, and virus titer were checked 8 d postinfection. E/T, effector/target.

PFU) of LCMV left in the spleen. In striking contrast, mice infected with the same doses ( $2 \times 10^5$  and  $2 \times 10^6$  PFU) of LCMV clones isolated from spleens of carrier mice made poor CTL responses and were unable to clear the virus infection. All four LCMV spleen isolates were present in high titers (3.8–5.2  $\log_{10}$  PFU) in the spleens of recipient mice at 8 d after infection. It should be noted that LCMV-specific CTL induced by Armstrong strain or by the LCMV spleen isolates were cross-reactive and killed syngeneic targets infected with either clone 13 or Armstrong with equal efficiency. In other experiments we tested the reactivity of CTL generated during infection with LCMV clones 1, 3, and 14 on targets infected with the homologous LCMV clones and found minimal killing (<20%) in all instances (data not shown). Thus, the data in Table VII showing limited CTL activity after infection with the LCMV spleen isolates represent fewer CTL and not the lack of appropriate targets. All four LCMV spleen isolates induced good LCMV-specific antibody responses. The amount of LCMV-specific antibody made after infection with the spleen isolates was equivalent to that induced by LCMV Armstrong. Thus, infection of N  $\rightarrow$  N mice with LCMV spleen isolates from carrier mice resulted in good antibody responses, poor CTL responses, and an inability to clear the virus. The same pattern of LCMV-specific immune responses and outcome of infection was seen in C + N  $\rightarrow$  N mice. These results suggest that the suppression of LCMV-specific CTL responses and the establishment of persistent infection by carrier spleen cells is due to the presence of genetic variants of LCMV in carrier mice. This was further tested by coinfecting N  $\rightarrow$  N mice with LCMV Armstrong and LCMV clone 13, and then checking CTL and antibody responses and the presence of virus (Table VIII). LCMV clone 13 selectively suppressed the CTL response and had no effect on the antibody response. Moreover, the CTL suppression was associated with an inability to clear the virus, and all N  $\rightarrow$  N mice infected with LCMV clone 13 or coinfecting with LCMV Armstrong and LCMV clone 13 became long-term carriers. High levels of infectious LCMV were present in the sera of these mice at 90 d after infection. Thus, the results presented in Tables VII and VIII show that active suppression of CTL responses by carrier spleen cells and the associated persistent infection are due to genetic variants of LCMV in spleens of carrier mice.

### Discussion

We have examined the interaction between LCMV and the host's immune system and defined conditions that lead to viral clearance or persistence. Our study establishes the following major points: (a) Spleen cells from carrier mice actively suppress LCMV-specific CTL responses of spleen cells from normal adult mice. (b) Suppression is specific for the CTL response and LCMV-specific antibody responses are not affected. (c) Suppression of CTL response is associated with the establishment of persistent infection. (d) Transfer of spleen or lymph node cells containing LCMV-specific CTL results in virus clearance and prevents establishment of the carrier state. (e) Suppression of CTL response is caused by genetic variants of LCMV in spleens of carrier mice. We propose that these LCMV variants selected during infection *in vivo* play a crucial role in the maintenance of the carrier state.

TABLE VIII  
*Suppression of CTL Response and Establishment of Persistent Infection by LCMV Clone 13, a Genetic Variant Isolated from Spleen of Carrier Mice*

Source of spleen cells transferred*	Challenge virus	LCMV-specific CTL in spleen			LCMV-specific antibody in serum	LCMV titer in serum (log <sub>10</sub> PFU/ml)				
		Percent specific <sup>51</sup> Cr release from BALB CL-7 (H-2 <sup>d</sup> ) targets (E/T, 50:1)				ELISA titer (log 2)	Days postinfection			
		Uninfected	LCMV Arm-strong infected	LCMV clone 13 infected	8		30	45	55	90
Normal	LCMV Armstrong	3	58	52	13.9	<1.6	<1.6	<1.6	NT <sup>‡</sup>	NT
		0	52	54	14.1	<1.6	<1.6	<1.6	NT	NT
		1	52	57	13.7	<1.6	<1.6	<1.6	NT	NT
Normal	LCMV Clone 13 <sup>‡</sup>	2	6	8	12.7	4.7	5.4	4.1	4.3	5.1
		0	0	0	14.6	4.3	5.3	4.2	4.6	4.3
		0	2	3	13.3	3.9	5.1	4.7	NT	NT
Normal	LCMV Armstrong + LCMV clone 13	3	7	12	13.3	4.0	5.4	4.8	5.3	4.7
		0	6	2	13.8	4.1	5.4	4.2	5.3	3.9
		1	8	7	12.9	3.8	5.1	5.0	5.3	5.1

\*  $5 \times 10^7$  spleen cells from adult BALB/c WEHI mice were transferred intravenously into normal adult BALB/c WEHI mice irradiated (600 rad) 1 d previously. At the same time recipient mice were infected intravenously with  $2 \times 10^5$  PFU of the indicated virus. Mice coinfecting with LCMV Armstrong and LCMV clone 13 received  $2 \times 10^5$  PFU of each virus. There were six mice in each group. Three mice from each group were sacrificed 8 d postinfection and checked for LCMV-specific CTL and antibody. The remaining mice in each group were eye-bled at the indicated times and the titer of infectious LCMV in the serum was determined by a plaque assay on Vero cell monolayers.

<sup>‡</sup> Not tested.

<sup>‡</sup> LCMV clone 13 was isolated from the spleen of 2-mo-old BALB/c WEHI carrier mice inoculated at birth with triple plaque-purified LCMV Armstrong.

The mechanism of CTL unresponsiveness in carrier mice and its role in the persistence of LCMV has been intensively studied (5, 6). Other investigators (17–19) have shown that carrier spleen cells actively suppress the induction of LCMV-specific CTL responses of normal adult mice. Dunlop and Blanden (19) showed that suppression is due to the high level of infectious virus in carrier spleen cells. Our studies confirm and extend these earlier observations by showing that suppression is due to genetic variants of LCMV. These earlier studies did not examine the effect of carrier spleen cells on LCMV-specific antibody responses or on the outcome of infection. We have shown that suppression by carrier spleen cells is specific for the CTL response and there is no inhibition of antibody response against LCMV. Most significantly, this study demonstrates the important biological consequence of this specific suppression, namely that this leads to viral persistence. Transfer of spleen or lymph node cells containing LCMV-specific CTL resulted in viral clearance and prevented establishment of the carrier state. Using a different protocol, Mims and Blanden (20) and Zinkernagel and Welsh (14) have shown the important role of LCMV-specific CTL in protecting mice from acute LCMV infections. Additionally, Byrne and Oldstone<sup>2</sup> found that cloned lines of LCMV-specific, H-2-restricted CTL effectively eliminated virus from spleens of acutely infected mice. Thus,

<sup>2</sup> Byrne, J. A., and M. B. A. Oldstone. Cloned cytotoxic T lymphocytes act *in vivo* to clear virus from mice acutely infected with lymphocytic choriomeningitis virus. Manuscript submitted for publication.

the results of the present study along with the other reports clearly establish that LCMV-specific CTL play a crucial role in clearing virus during acute infection and in preventing the establishment of persistent infection.

The genetic variants of LCMV that suppress CTL responses were isolated from spleens of 2-mo-old carrier mice infected at birth with triple plaque-purified LCMV Armstrong. We have found that 10 of 10 LCMV clones derived from that original wild-type LCMV Armstrong induced potent CTL responses (R. Ahmed, unpublished data). Thus, the original inoculum was homogeneous in this respect, and it is most likely that LCMV variants that suppressed CTL responses emerged during the infection *in vivo*. Other investigators (21, 22) have reported the presence of LCMV variants with different biological properties and plaque phenotypes in the brains and livers of carrier mice. The unique aspect of our studies is the dramatic difference we have identified in LCMV-specific CTL responses generated by LCMV variants isolated from spleens of carrier mice compared with those generated by the wild-type LCMV initially used to infect these mice. The variants suppressed CTL responses, leading to the important biological consequence of virus persistence. In striking contrast, wild-type LCMV induced a potent CTL response and the infection was rapidly cleared. Our results show that immunocompetent mice can become persistently infected when they are challenged with the appropriate LCMV variant. N → N mice infected with clone 13 were unable to eliminate the virus infection and became long-term carriers. Such mice have now been followed for 7½ mo postinfection and contain high levels of infectious virus in the serum and all major organs tested. Preliminary results indicate that adult immunocompetent BALB/c WEHI mice infected with LCMV variants make poor CTL responses and are unable to clear the virus for at least 6 wk postinfection (R. Ahmed, unpublished data). With our finding that LCMV variants can persist in adult mice, a model is now available for assessing the initiation of virus-induced autoimmune disease, the formation and deposition of immune complexes, and the imbalance of differentiated functions with altered homeostasis in adults infected with virus. These states have previously been described only with the LCMV infections initiated *in utero* or at birth (5, 6, 35). Further, our data indicate that immunocompetent adults can be at risk for developing a persistent infection when exposed to the operational variant.

The ability of several viruses, especially those with RNA genomes, to undergo rapid mutation during both acute and persistent infections has been amply documented (23–26). The majority of these studies have examined the evolution of viruses in tissue culture cells, and there have been relatively few studies analyzing genetic variation in viruses during replication in their natural hosts (23, 24). Selection of antigenic variants of visna virus during persistence in sheep, and of equine infectious anemia (EIA) virus in horses, has been described (27, 28). However, the role of these antigenic variants of visna virus and EIA virus in persistence and pathogenesis is unclear. The generation of LCMV variants that suppress CTL responses represents an example of a biologically meaningful selection. The LCMV-specific CTL response is important not only in virus clearance, but also in the immunopathologic process causing lethal acute choriomeningitis of adult immunocompetent mice (29, 30). Suppression of CTL

responses may, therefore, benefit both the host and the virus. If so, the selection of LCMV variants that suppresses CTL responses could represent a remarkable example of virus-host evolution towards balanced pathogenicity. Other examples of similar symbiotic relationships include the persistence of reovirus in L-929 cells and myxoma virus infections in rabbits (31, 32).

One must consider whether the markedly low level of CTL activity detectable in mice infected with the LCMV variants (or carrier spleen cells) represents dilution of CTL as a result of disseminated infection and subsequent recruitment of LCMV-specific CTL from the spleen to other infected organs (33, 34). Although such a dilution effect cannot be completely ruled out, the following observations suggest that low CTL activity detectable after infection with the LCMV variants is due to suppression of CTL responses. (a) CTL activity is low not only in the spleen but also in the inguinal, mesenteric, and axillary lymph nodes. (b) Virus is not cleared for at least several months, suggesting that the immune response generated during the infection is inadequate (i.e., suppressed). (c) The transfer of LCMV-specific CTL results in clearance of viral infection. Also, the transfer of spleen cells from adult immune mice (30–60 d after infection with LCMV Armstrong) results in the generation of CTL responses and subsequent elimination of virus (R. Ahmed, unpublished data). Thus, when LCMV-specific CTL activity is present, the virus infection is cleared. (d) Mice infected with the LCMV variants or carrier spleen cells cannot generate secondary LCMV-specific CTL after stimulation *in vitro*. Hence, no detectable memory CTL are present in the spleens or lymph nodes of these mice (R. Ahmed, unpublished data). All these observations strongly suggest that infection of mice with genetic variants of LCMV isolated from carrier mice results in suppression of LCMV-specific CTL responses. In fact, we have preliminary data indicating that the LCMV variants can suppress the generation of primary LCMV-specific CTL responses *in vitro* (R. Ahmed, unpublished data). A final point to emphasize is that suppression of CTL responses and the dilution effects due to disseminated infection are not mutually exclusive events. Both may occur simultaneously and contribute to an inefficient CTL response and failure to clear the infection.

How do the LCMV variants suppress CTL responses? One possibility is that these variants are lymphotropic and infect LCMV-specific CTL. LCMV is not an overtly cytolytic virus and it is unlikely that infection would kill the T cell. However, functional inactivation of CTL may occur due to selective inhibition of differentiated functions. Oldstone et al. (35) have shown that infection of growth hormone-producing cells by LCMV turns off the differentiated function (i.e., growth hormone production) but does not affect the vital functions (i.e., host macromolecular synthesis and replication). Conceivably, infection of LCMV-specific CTL by the LCMV variants may inhibit their differentiation into cytolytic effector cells capable of clearing the viral infection. Another possible mechanism of suppression is based upon the findings of Fink et al. (36) and Rammensee et al. (37) that CTL responses can be inhibited if antigen is inappropriately presented on T cells. Thus, infection of T cells by the LCMV variants and the subsequent expression of viral proteins on the membrane of these infected cells may deliver a negative signal and suppress the induction of the LCMV-specific CTL response. A critical difference between these two possible mechanisms of



suppression is that the first requires infection of a specific population of T cells, i.e., LCMV-specific CTL, whereas the second involves infection of any T cell. A third mechanism for CTL dysfunction is the presence of LCMV, or its antigens, in high concentration in the environment of the responding CTL. Lamb et al. (38) showed that influenza virus-specific helper T cell clones can be rendered unresponsive by high concentrations of specific antigen; suppression of CTL responses after infection with large doses of LCMV has also been reported (39, 40). Our preliminary results indicate that LCMV variants replicate to higher levels than wild-type LCMV Armstrong in spleens of infected mice (R. Ahmed, unpublished data), supporting this possibility. Our speculations on the mechanism of CTL suppression consider only the direct effects on LCMV-specific CTL. Equally possible is that suppression may follow some effect of LCMV variants on the macrophages or helper T cells that may be required to generate LCMV-specific CTL responses. The next steps are to sort out these possible mechanisms of CTL suppression and to characterize the structural differences between the variant virus that causes CTL suppression and the wild-type virus that induces CTL activation.

### Summary

We studied the mechanism of lymphocytic choriomeningitis virus (LCMV) persistence and the suppression of cytotoxic T lymphocyte (CTL) responses in BALB/c WEHI mice infected at birth with LCMV Armstrong strain. Using adoptive transfer experiments we found that spleen cells from persistently infected (carrier) mice actively suppressed the expected LCMV-specific CTL response of spleen cells from normal adult mice. The suppression was specific for the CTL response and LCMV-specific antibody responses were not affected. Associated with the specific CTL suppression was the establishment of persistent LCMV infection. The transfer of spleen or lymph node cells containing LCMV-specific CTL resulted in virus clearance and prevented establishment of the carrier state. The suppression of LCMV-specific CTL responses by carrier spleen cells is not mediated by a suppressor cell, but is due to the presence of genetic variants of LCMV in spleens of carrier mice. Such virus variants selectively suppress LCMV-specific CTL responses and cause persistent infections in immunocompetent mice. In striking contrast, wild-type LCMV Armstrong, from which these variants were generated, induces a potent CTL response in immunocompetent mice and the LCMV infection is rapidly cleared. Our results show that LCMV variants that emerge during infection *in vivo* play a crucial role in the suppression of virus-specific CTL responses and in the maintenance of virus persistence.

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