INHIBITION OF IMMUNOLOGIC INJURY OF CULTURED CELLS INFECTED WITH LYMPHOCYTIC CHORIOMENINGITIS VIRUS: ROLE OF DEFECTIVE INTERFERING VIRUS IN REGULATING VIRAL ANTIGENIC EXPRESSION*

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Persistent virus infections are characterized by the inability of the host's immune system to eradicate the infectious agent. This may result from a failure or dysfunction of the immune response or to a viral property which allows the infectious agent or infected cell to escape immunosurveillance.

For example, Scrapie, Kuru, and Jacob-Creutzfeldt agents may not elicit a detectable immune response (1, 2), whereas other viruses such as mouse thymic agent, lactic dehydrogenase virus, or measles virus may alter the immune response owing to their replication in or destruction of lymphoid cells [thymus-derived (T) and bone marrow-derived (B) lymphocytes and/or macrophages] which comprise the immune system (reviewed in references 3 and 4). Lack of or diminished response to viral antigens may perhaps also occur after high antigen loads or by infection before maturation of the immune system (5, 6).

In contrast, certain viruses like herpes simplex, cytomegalo, and measles persist despite a competent and vigorous host immune response. Here cells bearing viral genetic information are not eliminated by immune attack mechanisms. This may occur when appropriate concentrations of immune reagents are not reached in certain organs, when the infected cell is not in the appropriate phase of its growth cycle (7, 8), or when there is insufficient expression of viral antigens on the cell's surface (9, 10). Specific anti-viral antibodies can strip viral antigens off the cell's plasma membrane, leaving a cell in which virus persists but which is resistant to lysis by either viral-specific lymphocytes (11) or by antibody (A)[1] and complement (C) (9). In addition, the virus itself may have properties which favor its persistence. This may be by integration of viral genetic information in the host genome (12–14), by selection of less virulent variants or temperature-sensitive mutants (15), or by generation of defective interfering (DI) virus (16–18). Virus-infected cells may release products, like interferon, that play a role in both viral and immune regulation (19–21).

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[1] *Abbreviations used in this paper*: A, antibody; DI, defective interfering; LCMV, lymphocytic choriomeningitis virus; MEM, Eagle's minimal essential medium supplemented with glutamine, antibiotics, and serum; MOI, multiplicity of infection; NP40, Nonidet P-40; PFU, plaque forming unit(s); S, standard; VSV, vesicular stomatitis virus.

Lymphocytic choriomeningitis virus (LCMV) infection of cultured cells is a convenient model in which to study both immune regulation and viral persistence. Parameters of immunologic-mediated injury of virus-infected cells by T lymphocytes (22), anti-viral A and C (23, 24), and antibody-dependent lymphocyte killing (24) have been described as has the natural state of LCMV persistence in vivo (25–28) and in vitro (29–30). After infection of cultured cells with LCMV, infectious or standard (S) virus is initially generated, followed in time by the production and release of DI virus without detectable S virus (29, 31). Cloning, superinfection, and immunofluorescence analyses indicate that most if not all persistently infected cells carry viral genetic information (29). Neither the generation of temperature-sensitive mutants (R. M. Welsh, unpublished data), the integration of viral genome (32, 33), nor the production of interferon (29, 31, 34, 35) has been detected in cultures of LCMV persistently infected cells.

To better understand virus persistence and escape from immune surveillance, we studied the susceptibility of LCMV-infected cells in both the acute and persistent stages of infection to immune cytolysis mediated by sensitized T lymphocytes and anti-LCM viral A and C. We noted that a significant number of persistently infected culture cells escaped killing by both sensitized T lymphocytes and by anti-viral A and C. The inability to lyse virus-infected cells correlated with DI virus inhibition of viral antigen synthesis. The likely importance of these findings in in vivo infection are discussed.

Materials and Methods

Virus, Cells, and Infection. The Armstrong strain of LCMV (36) was used throughout these studies. L-929 cells (*H-2k*) were propagated in Eagle's minimal essential media supplemented with glutamine, antibiotics, and 10% fetal calf serum (MEM). N115 (murine neuroblastoma clone 115, *H-2a*) (37) and BHK 21/13S (36) cells were grown in Dulbecco's media as described. For acute infection, a virus stock containing 4×10^7 plaque-forming units (PFU)/ml was prepared after three dilute passage transfers in L-929 cells to avoid large contamination with DI LCMV. Stock virus was diluted 1:33, and 1 ml was added to each tissue culture Petri plate (Falcon no. 3002; Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) containing 1–1.5 $\times$ 10^6 L-929 cells (multiplicity of infection (MOI) of approximately 1). After adsorption at 37°C for 1½–2 h the virus inoculum was decanted, and the monolayers on the cultured plates were replenished with 5 ml MEM. Multiple identical samples were run in each experiment to ensure that points from different time periods represented data derived from separate Petri plates. At designated times, culture fluids and cells were harvested and analyzed by the method listed below. Acutely infected cultures were maintained for 7 days before the first passage and passaged weekly thereafter. Persistently infected cells were routinely passaged weekly and assayed for viral antigenic expression and infectivity as previously reported (29, 37). To initiate persistent infection, cultures were infected with L-929 cell-grown LCMV which had been passaged through a 0.22 μm filter. The L-929, N115, and BHK culture lines persistently infected with LCMV were maintained in this laboratory for over 2 yr.

S LCMV was titrated by the BHK agarose suspension plaque assay (38) and virus-infected cells were assayed by infective center assay on the same cells (39). The assay for DI LCMV was the infective center inhibition assay (16); briefly, monolayers of L-929 cells were exposed to an interfering dose of virus for 1½ h and then challenged with S LCMV. After an additional 1½ h incubation the cells were washed several times and then plated onto the BHK cell plates. Control preparations received MEM in place of the interfering inoculum. The amount of DI LCMV was calculated by the relative inhibition of infective center formation.

Lymphocyte-Mediated Killing. Uninfected and LCMV-infected L-929 cells were radiolabeled by mixing 5×10^5 cells with 20 μCi of ^{51}Cr (New England Nuclear Corp., Boston, Mass.) in minimum volume. After 40 min incubation at 37°C in 5% CO_2, the cells were washed five times with 10 ml of MEM and resuspended in MEM to a final concentration of 1×10^5 cells/ml. The labeled cells in 100-μl samples were distributed into individual microtiter wells (Falcon plate no. 1020; Falcon Plastics, Div. of BioQuest) (10^4 cells per well). 3 h later spleen cells obtained

from C3H/St (*H-2k*) or A/J (*H-2a*) mice were added. Both murine strains are syngeneic with L-929 cells in the *K* region of the *H-2* locus (40). Spleens were removed from three to five uninfected mice or mice injected 5–7 days previously by the intraperitoneal route with 3×10^4 PFU of LCMV. The pooled spleens were gently squeezed with forceps, liberating the cells, and aggregates were removed by filtration through cheesecloth. Erythrocytes in the preparation were lysed by treatment with 0.83% NH_4Cl for 5 min. After two washes with MEM, the spleen cells were added to ^{51}Cr-labeled target cells at a constant ratio of 100:1. Varying amounts of sensitized lymphocytes were used by mixing them with lymphocytes obtained from normal mice (100:0, 75:25, 50:50, 25:75 and 0:100). Samples of target cells treated with MEM and 1% Nonidet P-40 (NP40) (Shell Chemical Co., New York) served as controls for spontaneous ^{51}Cr release and maximum ^{51}Cr release, repectively. Incubations lasted for 16 h at 37°C. Assay plates were then centrifuged at 600 *g* for 5 min, and 100 ml of supernatant fluid was removed from each well, placed into a glass tube, and counted in a Searle automatic gamma counter (Amersham/Searle Corp., Arlington Heights, Ill.). Determinations were run in quintuplicate. The percent specific ^{51}Cr release was calculated by the following equation:

$$\% \text{ specific release} = \frac{(\text{cpm test sample} - \text{cpm MEM control})}{(\text{cpm NP40 control} - \text{cpm MEM control})} \times 100.$$

Identification of T lymphocytes as cells that killed virus-infected targets was determined by depletion experiments in which T cells were removed from the lymphocyte population by the addition of A to theta antigen and C (24).

A-Mediated C-Dependent Killing. In most experiments fresh sera before or after immunization of guinea pigs with purified LCMV grown in BHK cells (36) served as the source of C or antiviral A and C, respectively. When necessary, sera were absorbed with L-929 and/or N115 uninfected cells to remove cross-reacting antibodies against cell membrane determinants (23, 41). Guinea pig IgG was prepared by ammonium sulfate precipitation and DEAE-cellulose chromatography. IgG (2 mg/ml) that was pure as determined by Ouchterlony and immunoelectrophoresis analysis was conjugated to fluorescein isothiocyanate or radiolabeled with ^{125}I by the chloramine-T method (41). Congenic antibodies to *H-2k* and to *H-2d* were a gift from Dr. Rolf Zinkernagel, Scripps Clinic and Research Foundation, La Jolla, Calif.

The ability of anti-LCM viral antibody in the presence or absence of C to kill virus-infected or uninfected cells was assayed by the microcytotoxicity method (42). Briefly, 6 μl of fresh antiserum (A and C source) and 1 μl of cell suspension (5×10^5/ml in MEM) were discharged under mineral oil onto a Moller-Coates plate (HTI Corp., Associated Biomedic Systems, Buffalo, N. Y.). In all experiments a control consisting of cells and heated serum (56°C, 30 min) was included. The plates were then incubated for 90 min at 37°C after which 1 μl of 5% eosin and then 1 μl of undiluted formaldehyde were added to each reaction mixture. Viability was assessed by using a binocular inverted phase microscope with an adjustable condensor (6-mm focal point) at a magnification of 200. When different sources of A and C were employed, the cells were incubated with 2 μl of A solution (previously heated at 56°C for 30 min) for 30 min at 37°C after which 6 μl of C was added. For each experimental point 200 cells were counted. Nonspecific background of cells never exceeded 5%.

Detection of C Receptors. C3b receptors were detected with antibody-coated sheep erythrocytes treated with rabbit serum deficient in the 6th component of C (EAC1423b cells). C3d receptors were detected with antibody-covered sheep erythrocytes treated with mouse serum deficient in the 5th C component (EAC1423d cells). The exact procedures for making these indicator cells, use of negative and positive controls, and detection of C3b and C3d receptors on cells have been detailed elsewhere (43, 44).

Immunofluorescence Assay. L-929 cells grown on cover slips in Petri dishes were infected with LCMV as described above. At designated time intervals the cells were fixed in ether alcohol, washed in 95% alcohol (28), and stained directly with monospecific guinea pig IgG conjugated to fluorescein isothiocyanate. After vigorous pipetting, the remaining cells in the Petri dish were suspended in phosphate-buffered saline, and a pellet of 5×10^5 living unfixed cells was stained with the same A conjugate (23). The percent of cells expressing viral antigens was calculated after counting 100–200 cells.

Quantitation of Surface Viral Antigens. The numbers of specific antibody molecules binding to viral antigens expressed on the surface of infected L-929 cells were determined as reported (41). In

brief, guinea pig IgG containing A to LCMV was labeled with ^{125}I while guinea pig IgG lacking A to LCMV was labeled with ^{131}I. Both preparations were absorbed against uninfected L-929 cells and deaggregated by ultracentrifugation before use. Varying concentrations of uninfected and infected L-929 cells (1×10^4, 5×10^4, 1×10^5, and 5×10^5) were mixed with both ^{125}I- and ^{131}I-labeled IgG preparations. In other assays increasing amounts of radiolabeled IgG were added to constant numbers of cells. By examining the saturation curves, an approximation of a maximal amount of antibody molecules binding per cell at antigen saturation levels was calculated (41).

DI LCMV Analysis and Assay. DI LCMV (assayed by infective center inhibition assay) without detectable S virus was isolated from culture fluids of persistently infected L-929, BHK, or N115 cultures. The density of L-929 cell-grown DI LCMV purified by methanol precipitation and a discontinuous sucrose gradient (36) was determined by sedimentation on an 11 ml 20–50% wt/wt continuous sucrose gradient centrifuged at 40,000 rpm for 16 h in a Beckman no. SW41 rotor (Beckman Instruments, Inc., Fullerton, Calif.). Individual samples were collected, and the densities were recorded using a refractometer. Each sample was then diluted 1:3 in growth MEM and 1.5-ml samples were added to L-929 monolayers to test for the presence of interfering material by infective center inhibition assay. Controls containing MEM and similar sucrose concentrations were also used as an interfering inoculum. The residual sucrose in each sample (17% sucrose highest in concentration) did not inhibit infective center formation. S LCMV was sedimented in a separate gradient in parallel, and fractions were collected and assayed for plaque formation.

Cultivation and Immunofluorescent Staining of Mouse Peritoneal Cells. Peritoneal cells were isolated from control and LCMV persistently infected BALB/c heterologous nude and SWR/J mice. These mice had been infected less than 24 h after birth by intracranial inoculation with 700 PFU of LCMV and had shed virus throughout their lives. Cells were obtained by washing the peritoneal cavity with 6 ml MEM lacking serum but containing 20 U/ml heparin (Lipo-Hepin; Riker Laboratories, Inc., Northridge, Calif.). In some instances mice were injected intraperitoneally with 1 ml of 10% thioglycollate broth (45) to recruit macrophages to the peritoneal cavity. Cells were directly placed on Petri dishes with glass cover slips, and after 1–2 h, the nonadherent cells were washed away with MEM. For immunofluorescent staining of LCMV antigens on the cell surface of the adherent cells, several modifications were made. The cells were suspended after incubation with 0.3% lidocaine HCl (Xylocaine; Astra Pharmaceutical Products, Inc., Worcester, Mass.) in MEM for 10 min. The suspended cells were stained with fluorescein-conjugated Fab fragments of the guinea pig anti-LCMV IgG molecule. Preparation of Fab from guinea pig IgG was by papain digestion and DEAE-cellulose chromatography procedure as described (46, 47). The method for staining the cytoplasms of adherent peritoneal cells was identical to that for the L-929 cells.

Results

Susceptibility of Acutely and Persistently Infected L-929 and N115 Cells to Immune Attack. After acute infection of L-929 cells with LCMV, the amount of infectious virus in the culture fluids peaked on the 2nd through 5th days (Table I). *De novo* S LCMV synthesis observed by changing the medium daily in the cultures was maximum at the 2nd and 3rd days. The number of cells producing S virus, as shown by infective center assay, correlated with the *de novo* virus synthesis. Precipitous drops in the percent infective centers occurred from day 3 (79%) to day 4 (14%) to day 5 (3%), even though S LCMV was still present in the culture fluid. Thereafter, the infectivity declined, only to rise and fall on subsequent passages at days 13, 21, and 29 postinfection. In previously reported studies, marked peaks and valleys in S virus synthesis occurred in similar cultures for up to 100 cell generations (29, 30). Persistently infected L929, N115, and BHK cells (infected for over 2 yr) released no detectable S virus into the culure fluid, and did not form infective centers when plated on uninfected cells (Table I). As judged by infective center assay, all persistently infected cells were resistant to LCMV superinfection, indicating viral information in every cell (29).

TABLE I

Kinetics of Antigenic Expression and Susceptibility of L-929 Cells Infected with LCMV to Immune-Specific Lysis

Day of infection	PFU per ml (log 10)	PFU/ml* with medium change	% Infective centers	% Cells with virus antigens‡		% Immune-specific lysis by:	
				Cytoplasm	Surface	A + C	Lymphocytes
1	5.5	5.9	48	5	4	<5§	31 ± 0.17‖
2	7.4	8.0	107	60	43	7	58 ± 0.91
3	8.3	7.5	79	98	85	27	67 ± 0.84
4	8.0	6.7	14	94	74	28	74 ± 1.7
5	7.9	5.7	3	88	30	5¶	59 ± 1.2
6	6.9	4.7	ND**	74	22	−¶	63 ± 0.45
7	6.4	3.8	ND	75	12	−¶	54 ± 1.2
13	1.0	ND	ND	ND	10	ND	42 ± 0.65
21	6.6	ND	ND	26	12	ND	38 ± 1.5
29	3.7	ND	ND	9	3	ND	17 ± 0.23
>720	<1.0	<1	<0.001	63	6	<5	46 ± 0.72
Uninfected	<1.0	<1	<0.001	<0.01	<0.01	<5	11 ± 0.53

* Medium changed daily and fresh medium added in order to determine *de novo* infectious virus synthesis and release.

‡ Determined by direct immunofluorescence using monospecific guinea pig IgG antibody to LCMV conjugated to fluorescein isothiocyanate. Over 200 cells counted per point.

§ % of cells killed determined by a microcytotoxicity assay. Number represents the mean value of over 200 cells counted.

‖ ^{51}Cr specifically released. Number represents the mean value from quintuplicate determinations ± 1 standard error of the mean. Effector spleen cells were harvested from syngeneic donor mice, C3H/St (*H-2k*) 6 days after primary challenge with LCMV. Ratio of effector to target cells was 100:1. See Materials and Methods.

¶ Number represents A-dependent C killing only, but A-independent C killing also occurred in these samples.

** ND, not determined.

The susceptibility of infected L-929 or N115 cells to immune attack during different stages of infection was examined by using the ^{51}Cr-release assay for lysis by immune lymphocytes and the microcytotoxicity assay for lysis by antibody to LCMV and C. Spleen cells from C3H/St or A/J mice (sensitized to LCMV) killed significant numbers of both acutely and persistently infected L-929 (Table I) or N115 (Table II) cells, respectively. In all five experiments performed using a variety of effector to target cell ratios and spleen cells harvested at varying times after sensitization, significantly fewer persistently infected cells were killed than acutely infected cells (Tables I and II). The average of five experiments indicated that >40% of the persistently infected cells escaped lysis by immunologically specific lymphocytes under those conditions. This relative inability of sensitized spleen cells to kill persistently infected cells carrying LCMV antigens occurred whether the targets were L-929 or N115 cells. Lysis of acutely and persistently infected target cells was mediated by T lymphocytes, since this killing was abrogated when sensitized spleen cells were treated with antibody to theta antigen and C.

Only acutely infected cells were killed by anti-LCMV viral A and C. Neither uninfected cells nor cells 1 day after acute LCMV infection nor persistently

TABLE II

Susceptibility of N115 Cells Infected with LCMV to Lysis by Virus-Specific Cytotoxic T Cells

Exp.	Day of spleen cells harvested*	Ratio effector to target cell	Percent ^{51}Cr release from target cells‡		
			Uninfected	Acute LCMV infection	Persistent LCMV infection
1	5	100:1	28 ± 0.25	44 ± 0.34	29 ± 0.21
	6	100:1	18 ± 0.52	95 ± 2.4	51 ± 1.9
	7	100:1	16 ± 0.47	87 ± 0.76	59 ± 0.75
2	6	100:1	15 ± 0.37	68 ± 2.0	44 ± 1.3
		75:1	14 ± 0.19	58 ± 1.0	35 ± 1.4
		50:1	10 ± 0.38	53 ± 0.70	38 ± 0.15
		25:1	8 ± 0.58	51 ± 0.39	28 ± 1.2
3	7	100:1	17 ± 0.34	75 ± 1.6	61 ± 0.67
		75:1	12 ± 0.40	72 ± 0.52	56 ± 0.60
		50:1	8.0 ± 0.042	76 ± 0.98	53 ± 0.59
		25:1	4.6 ± 0.052	65 ± 1.4	46 ± 0.30
		10:1	1.5 ± 0.013	34 ± 0.40	32 ± 0.55
		5:1	0.4 ± .0086	21 ± 0.24	17 ± 0.37

* Syngeneic A/J mice were infected intraperitoneally with 2×10^5 PFU of Me70 strain LCMV 5, 6, or 7 days before assay.

‡ For acute infection, N115 cells were infected with MOI of 1 and used on the 2nd day after infection. Persistently infected cells had been infected with LCMV for over 720 days at the time of the assay. Numbers represent the mean of quintuplicate determinations ± the standard error of the mean.

infected cells could be lysed by anti-viral A and C. Maximal lysis usually occurred on the 2nd to 4th day after acute infection (Table III). In several experiments the maximum amount of specific A-mediated C lysis was 20–35%. In addition, in five of five experiments we found that rarely on the 2nd but usually by the 3rd to 6th day after infection, a C source devoid of antibodies to LCMV lysed virus-infected cells (Table III). This C lysis independent of antibody to LCMV occurred with guinea pig, rabbit, and human C sources. By the 7th to 8th day after infection and thereafter lysis by C alone did not occur. This lysis was not associated with the appearance of C3 receptors on the virus-infected cells since EAC1423b or EAC1423d indicator cells did not bind to the LCMV-infected L-929 cells. Further, this possibly was not related to the binding of nonspecific A(s) to uncovered determinants on the infected cell's membrane as ^{131}I control antibodies did not bind to these cells (Table IV). Whereas persistently infected cells were not lysed by anti-LCMV A and C, they were readily killed by specific A to their major histocompatibility determinant and C (Table III). Using the same C source, similar numbers of target cells and twofold serial dilutions of A to *H-2k*, we found that the 50% A lysis titers of uninfected, acutely infected (day 2), and persistently infected L-929 cells were 1:26, 1:23, and 1:40, respectively. These results indicated that (*a*) persistently infected cells could be lysed by A and C and (*b*) that the amounts of *H-2k* present on surfaces of

TABLE III
Susceptibility of L-929 Cells Infected with LCMV to Lysis by Virus-Specific A and C

Cultured cells	Culture no.	Percent of cells lysed by:*				% Cells binding EAC rosettes	
		C alone	Anti LCMV A + C	C alone	Anti-H-2 A + C	C3b	C3d
Uninfected							
	1	13	14	13	98	1	0
	2	11	3	16	100	<1	0
Acute infection							
Day 1		15	22	14	98	2	<1
Day 2		25	58	22	100	0	0
Day 3		49	68	46	100	3	3
Day 4		54	58	ND	ND	0	0
Day 5		86	84	ND	ND	2	0
Persistent infection							
>720 days*	1	16	11	15	99	0	0
>720 days‡	2	13	10	13	100	0	0

* Microcytotoxicity assay was described in Materials and Methods. A alone killed <15% of cells. C, complement source plus MEM. These are representative results of one of three experiments. Control of A to *H-2d* did not lyse L-929 (*H-2k*) cells. Rosetting and anti-viral A experiments were run concurrently. In all experiments L-929 cells were infected acutely with stock LCMV at MOI of 1.

‡ Raji cell (with C3b and C3d receptors) control run concurrently showed >75% binding EAC3b and >60% binding EAC3d indicator cells (43, 44).

uninfected, acutely infected, and persistently infected cells did not vary significantly.

To determine the relationship between viral antigenic expression and the susceptibility of virus-infected cells to immune cytolysis, cells were examined for their abilities to bind fluorescein-labeled and radiolabeled A to LCMV. The binding of anti-viral A labeled with fluorescein (Table I) or ^{125}I (Table IV) to the cell membrane peaked on the 3rd and 4th day postinfection. Thereafter, the amount of viral antigenic expression on the membrane precipitously declined, even though significant quantities of S LCMV remained in the culture fluid (Table I). By 7 days postinfection, the amount of ^{125}I-A binding to infected cells was the same as that for uninfected controls. In contrast, most cells in the culture continued to express cytoplasmic viral antigens (Table I). Although 63% of the persistently infected cells displayed cytoplasmic viral antigens by fluorescence, there was little antigenic expression on their plasma membranes. Only trace amounts of LCMV antigens appeared on the surfaces of 6% of the persistently infected cells by fluorescent-binding assay, and these cells were indistinguishable from uninfected controls when the radiolabeled A-binding assay was used (Table IV). Knowing the specific activity of the ^{125}I-labeled antibody to LCMV, we calculated that for A-mediated C lysis to occur, the binding of approximately 5×10^5 A molecules per target cell was required.

Characteristics of LCMV Released by Persistently Infected L-929 Cells. As observed with other cell types including N115 and BHK (29, 31, 32, 37), L-929

TABLE IV

Binding of LCMV Antibodies to Surfaces of L-929 Cells Infected with LCMV

Cell conditions	No. cells* in assay	^{131}I (non-LCMV A)		^{125}I (LCMV A)		No. specific A molecules bound
		CNTS‡	% Bind	CNTS‡	% Bind	
Acute infection						
Day 1	5×10^5	2,187	0.06	3,710	0.06	1×10^5§
	5×10^4	1,928	0.06	2,829	0.05	
Day 2	5×10^5	1,330	0.04	5,389	0.10	2×10^5
	5×10^4	1,474	0.05	3,570	0.06	
Day 3	5×10^5	1,762	0.05	16,269	0.30	5×10^5
	5×10^4	1,607	0.05	4,528	0.08	
Day 4	5×10^5	1,595	0.05	22,847	0.40	7×10^5
	5×10^4	1,763	0.05	5,625	0.10	
Day 5	5×10^5	1,815	0.06	9,493	0.16	3×10^5
	5×10^4	1,527	0.05	3,769	0.06	
Day 6	5×10^5	1,412	0.04	5,879	0.10	2×10^5
	5×10^4	1,525	0.05	3,323	0.06	
Day 7	5×10^5	1,502	0.05	6,169	0.11	2×10^5
	5×10^4	1,720	0.05	3,402	0.06	
Persistent infection	5×10^5	1,414	0.05	3,726	0.06	1×10^5§
	5×10^4	1,481	0.05	3,425	0.06	
Uninfected	5×10^5	2,063	0.06	3,013	0.05	1×10^5§
	5×10^4	2,154	0.07	3,555	0.06	

* Cells used in assays were at dilutions of 5×10^5, 1×10^5, and 5×10^4 with results shown for two concentrations.

‡ Radioactive counts (CNTS) corrected for background and crossover.

§ Background binding levels.

cells persistently infected with LCMV were completely resistant to superinfection with S LCMV but were totally permissive to vesicular stomatitis virus (VSV) infection as judged by release of infectious virus. These persistently infected cells released virions that by electron microscopy were morphologically similar to S LCMV (P. Lampert, personal communication). The cells released an interfering component which prevented S LCMV synthesis and infective center formation, and this interfering activity was specifically blocked by antibodies to LCMV. Under the same conditions of sedimentation in sucrose, the density of the interfering activity (DI LCMV) was 1.15–1.165 g/cm^3 as compared to 1.165–1.175 g/cm^3 for S LCMV. The interfering component obtained from supernates of persistently infected BHK and N115 cells also had lower densities (1.15–1.165 g/cm^3) than S LCMV. The culture fluids from persistently infected L-929, N115, and BHK cells produced no detectable LCMV antigens when inoculated onto susceptible cells, nor did those inoculated cells release a detectable interfering component. The resistance of these treated cell cultures to superinfection by LCMV waned after 24 h. These experiments indicated that the persistently infected cells released a DI virus.

Inhibition of S LCMV Antigen and PFU Synthesis by DI LCMV. Cells exposed to DI LCMV isolated from either L-929, N115, or BHK persistently infected cultures expressed no viral antigens by immunofluorescence at 24 and

TABLE V

(A) Prevention of Viral PFU and Antigen Production by DI LCMV and (B) inhibition of PFU Production on Second Transfer

Exp. A*				Exp. B‡		
DI virus dilution	S LCMV	% Cells expressing cytoplasmic antigens	Log_{10} PFU per culture	Interfering inoculum	Log_{10} PFU yield without challenge	Log_{10} PFU yield with LCM challenge
Neat DI	+	1.9	3.2 (A)	A	2.3	4.8
1:2 DI	+	3.8	4.3 (B)	B	2.3	4.8
1:4 DI	+	8.4	5.0 (C)	C	3.0	3.4
1:8 DI	+	19.0	5.3 (D)	D	3.3	3.7
1:16 DI	+	19.4	5.8 (E)	E	3.7	4.7
1:32 DI	+	27.1	6.1 (F)	F	4.0	4.6
MEM	+	35.6	6.2 (G)	G	4.2	4.4
MEM only	−	0	<1 (H)	H (control)	<1	4.6

* L-929 cells were exposed to dilutions of a DI LCMV stock prepared from a 120 times concentration of medium from persistently infected L-929 cells. After 2 h, S LCMV (MOI = 1) was added to the cultures. After 2 h the inocula were decanted, and MEM was placed on the cells. At 16 h postinfection, the medium was titrated for PFU and the cells were fixed and stained with immunofluorescent antibody to LCMV.

‡ Culture fluid samples representing the 16-h harvests from Table V A (samples A-H) were exposed to L-929 cells for 2 h. The interfering inocula were decanted, and S LCMV was added to the cells at a multiplicity of 2 PFU per cell. After 2 h the inoculum was removed and medium replenished. The culture fluids were harvested 24 h later and titrated for infectivity.

48 h after infection. In contrast, cells exposed to S LCMV displayed both cytoplasmic and cell surface viral antigens. When cells were first incubated with DI LCMV from either of the above cultures and then challenged with S LCMV, the synthesis of viral antigens was markedly inhibited. The inhibition of viral cytoplasmic antigen synthesis was dose dependent and increased with increasing concentrations of DI LCMV (Table V A). Table V A demonstrates that increasing doses of DI LCMV isolated from L-929 cells reduced the number of cells expressing antigens in a linear manner and lowered the PFU yield logarithmically. Under conditions of DI virus treatment that reduced the number of fluorescent cells by a factor of 20, the number of PFU produced was reduced by a factor of 1,000. In addition to indicating that DI virus at high concentrations blocks viral antigen synthesis, this experiment also suggests that much of the antigen present in the DI virus-treated cells is not released in the form of infectious S LCMV. Thus, either the antigen is released in a defective viral or protein form, or else the antigen is not released from the cell at all. To determine whether the DI plus S virus-treated cells synthesized and released an interfering component, L-929 cells were exposed to the culture fluid harvests from the experiment in Table V A and then challenged with S LCMV at a MOI of 1. Samples C and D, derived from a harvest of cells treated with 1:4 and 1:8 dilutions of DI virus, significantly inhibited the synthesis of challenge S LCMV (Table V B). Samples derived from stocks originally receiving more DI virus (A

TABLE VI

Defective Interfering Virus Regulates Expression of LCMV on Cell's Plasma Membrane

Treatment of L-929 cells		Assay at 24 h for:		
First	Second	Infectivity (log_{10} PFU/ml)	% Cells with surface viral antigens	% ^{51}Cr released on addition of sensitized lymphocytes
MEM	MEM	0	0	7 ± 0.18
DI LCMV*	MEM	0	0	7 ± 0.050
MEM	S LCMV	7.5	33	66 ± 0.15
DI LCMV	S LCMV	5.1	0	10 ± 0.55

* Supernatant fluids were obtained from BHK cells persistently infected with LCMV and concentrated 120 times (DI LCMV). Cells were exposed to DI LCMV (or MEM) for 1½ h and then challenged with MEM or S LCMV (MOI = 1) for 1½ h. Surface antigens were measured by immunofluorescence.

and B) and less DI virus (E and F) did not interfere with S LCMV synthesis. This indicates that under conditions of infection with both S plus DI virion types, S LCMV but little interfering component is produced by 24 h when small amounts of DI virus are used (E and F); at higher DI virus concentrations there is a reduction in the synthesis of S LCMV but an interfering component is produced (C and D); at the highest concentrations of DI LCMV there is little production of either S LCMV or an interfering component (A and B).

DI LCMV also blocked the expression of cell surface viral antigens. As detected by immunofluorescence 24 h after acute infection, 33% of L-929 cells exposed to S LCMV expressed surface viral antigens, but no cells treated first with DI LCMV did so (Table VI). The yield of S LCMV was reduced by over two logs by the DI LCMV in this experiment, and the treated cells were resistant to sensitized spleen cell-mediated cytotoxicity (Table VI). These same spleen cells readily lysed targets infected with S LCMV only. Again, the inhibition of viral antigen synthesis by DI virus depended on the concentration of DI virus in the inoculum, as some DI virus preparations which reduced the yield of S LCMV by over one log did not render cells resistant to spleen cell killing. Once initiated, viral antigen synthesis, particularly detectable at the cell surface, could be arrested by DI virus treatment. L-929 cells were infected with S LCMV at a multiplicity of 5 PFU per cell, and allowed to incubate for 4 h. They were then treated with different concentrations of DI LCMV isolated from persistently infected L-929 cells. At 24 h postinfection the cells were examined by immunofluorescence for cytoplasmic and cell surface viral antigens. The DI virus treatment did not substantially reduce the number of cells expressing cytoplasmic viral antigens (S LCMV, 66%; S plus DI LCMV, 55%), although the intensity of the fluorescent stain was clearly diminished in the DI virus-treated samples. The number of cells expressing the characteristic bright fluorescent pattern for LCMV was greatly diminished in DI virus-treated samples (S LCMV, 12%; S plus DI LCMV, 2%); some of the S plus DI LCMV-treated cells had very light speckles of immunofluorescence, but even when these were taken into account, the frequency of cells with surface staining was still lower than in the S LCMV-infected cultures (15% vs. 8.8%). Hence, DI LCMV was capable of preventing antigen synthesis in cells infected with S virus, and this was directly

TABLE VII
*Accumulation of Interfering Component (DI Virus) During Acute LCMV Infection in L-929 Cells**

Day after initiating infection	PFU per ml (log 10)	% Infective centers‡		24-h yield $\log_{10}$ PFU/ml	
		Undiluted	Diluted 1/10	Undiluted	Diluted 1/10
2	7.4	26.0	7.0	6.8	6.6
3	8.3	12.0	15.0	5.8	6.7
4	8.0	7.3	14.0	5.7	6.6
5	7.9	4.4	15.0	5.7	6.6
7	6.4	1.1	ND§	5.9	ND
21	6.6	37.0	ND	6.6	ND

* Cell monolayers were infected with LCMV (MOI = 1) and the culture fluid was sampled daily and titrated for PFU. The collected culture fluids were then inoculated onto L-929 cells. 1 h postinfection these cells were assayed for infective center formation. The remaining cells were reseeded onto Petri dishes and incubated for 24 h under MEM, after which the culture fluids were titrated.

‡ Culture fluids used undiluted or diluted 10-fold for assay system.

§ ND, not determined.

associated with the inability of immune reagents to lyse virus-infected cells (Table V and VI).

Production of an Interfering Component During Acute LCMV Infection. To determine whether the viral antigen shutdown during acute infection in vitro might be associated with production of DI LCMV and its interference with LCMV antigenic expression, culture fluids after the 2nd day of the acute infection were collected and studied. Such fluids obtained on days 3–7 after infection formed infective centers poorly when compared to samples obtained 21 days after acute infection (Table VII). Similarly, relatively few PFU formed in the culture fluid of L-929 cells inoculated with these same samples. Dilution of samples from days 3, 4, and 5, but not day 2, before inoculation onto L-929 cells enhanced infective center formation and increased the 24-h yield 10-fold. When the day 4 and day 2 harvests were mixed and inoculated onto L-929 cells, there consistently were 50–80% fewer infective centers formed than with cells treated with day 2 preparations only. The interference was not medium dependent, as the interfering component could be pelleted at 105,000 *g* for 60 min in an ultracentrifuge and resuspended in fresh MEM. These experiments indicate that an interfering component was present in the virus stock harvested on days 3–7 after infection.

To determine whether the interfering component was made *de novo* or was a stable by-product of acute viral infection which accumulated in the media associated with a relatively fast inactivation of S LCMV, culture fluids of infected cells were changed daily, and analyzed for their abilities to block infective center formation. Under these conditions the activity of the interfering substance was again observed on the 3rd and 4th day after instituting an acute infection. *De novo* synthesis of an interfering component on days 5 and 6 postinfection was not detected (without concentration), likely reflecting the culture's shutdown in surface antigen production. In parallel, culture fluids obtained from the day 4 harvest, undiluted or diluted 10-fold, were added to L-929 cell monolayers and incubated for 16 h. Several experiments showed that the diluted inocu-

TABLE VIII
Immunofluorescent Staining of Peritoneal Cells from LCMV-Carrier Mice

Mice		% Positive cells	
Strain	No.	Cytoplasm	Surface
nu/+ (BALB/c)	1	73	7.6
	2	71	22
	3	22	17
SWR/J	1	35	16
	2	85	50
	3	65	21

Adherent peritoneal cells from 8- to 14-mo-old mice were stained with fluoresceinated IgG (cytoplasm) or IgG Fab (surface) containing antibody to LCMV.

lum produced at least as many and sometimes twice the number of fluorescing cells as the undiluted inoculum (e.g. undiluted inoculum, 15% fluorescent cells; 10-fold diluted inoculum, 25% fluorescent cells), indicating that the interfering component in the culture fluid blocked antigen synthesis by the S virus also present in the culture fluid.

Antigenic Expression on Cells from Persistently Infected Mice. Adherent peritoneal cells isolated from LCMV persistently infected SWR/J or nu/+ (on a BALB/c background) mice resembled persistently infected cells in culture. As judged by immunofluorescence significantly more cells expressed cytoplasmic antigens than surface viral antigens (Table VIII). The relative frequencies of cells with viral antigens varied markedly between individual mice. Peritoneal cells isolated from persistently infected mice released low numbers of PFU into the culture fluid. Very few (0.1%) of those cells scored as infective centers, and the cells were refractory to superinfection by S LCMV (Table IX). Addition of S LCMV to a culture of peritoneal cells from LCMV-carrier mice did not increase the number of fluorescent cells in the culture. In contrast, peritoneal cells harvested from uninfected mice and inoculated with S LCMV release PFU into the culture fluid, scored high as infective centers (20%) and produced viral antigens. Although searched for, only sporadically was an interfering component against LCMV synthesis found in the culture fluid of peritoneal cells from LCMV-carrier mice. Owing to the low yields of the interfering component, it was not characterized when found.

DI LCMV produced in persistently infected BHK cells can interfere with S LCMV synthesis in adherent peritoneal cells. Table X shows that the infective center number of peritoneal cells infected with S LCMV was significantly reduced by pretreatment with DI LCMV.

Discussion

The concentration of LCMV antigens on the surface of infected cells is high during the early stages of infection but is significantly reduced as the infection continues. This dampening or modulation of antigenic expression is a function of the virus infection per se and occurs in the absence of added antibody. After the

TABLE IX
Inhibition of LCMV Synthesis in Mouse Peritoneal Cells

Source of cells	Superinfection with S LCMV	% Fluorescent cells (cytoplasm)	% Infective centers	Log_{10}/ml PFU yield
Uninfected mouse	No	0	ND*	ND
Uninfected mouse	Yes	51	20.0	4.3
LCMV-carrier mouse	No	56	0.1	2.0
LCMV-carrier mouse	Yes	41	0.3	3.5

Adherent peritoneal cells from SWR/J normal or LCMV-carrier mice were superinfected with S LCMV (at a multiplicity of 5 PFU per cell) and incubated for 48 h. Cells were then stained with immunofluorescent antiserum to LCMV and then titrated for infective centers and release of PFU into the culture fluid.

* ND, not done.

TABLE X
DI LCMV Inhibits S LCMV Synthesis in Mouse Peritoneal Cells

Cell	Interfering inoculum	Challenge virus	% Infective centers	Log_{10}/ml PFU yield (24 h postinfection)
Peritoneal	MEM	S LCMV	0.77	3.3
Peritoneal	DI LCMV	S LCMV	0.15	2.5
L-929	MEM	S LCMV	3.0	3.7
L-929	DI LCMV	S LCMV	0.05	1.8

Peritoneal cells from normal SWR/J mice and L-929 cells were exposed to DI LCMV concentrated 120 times from the culture fluid of persistently infected BHK cells. Cells were then challenged with S LCMV at a multiplicity of 0.3 PFU per cell. Infective centers were measured immediately after S LCMV adsorption, and PFU were titrated 24 h after incubation.

infection of L-929 cells with LCMV at a MOI of 1, the amount of LCMV cell surface antigens peaked on the 2nd to 4th day and then dropped precipitously, and cells persistently infected with LCMV for over 2 yr displayed low quantities of viral antigens on their plasma membrane surfaces. In contrast to these differences in expression of surface viral antigens, the majority of cells during both acute and persistent infections had detectable viral antigens in their cytoplasms. These antigenic characteristics of persistently infected cells have been observed previously (32, 48).

The susceptibility of LCMV-infected cells to immune lysis by anti-LCMV A and C directly paralleled the presence and concentration of LCMV antigens on the surface of the infected cells. On the 2nd to 4th day postinfection, viral antigenic expression was maximal and A-mediated C-dependent lysis occurred. Persistently infected cells, which had little cell surface viral antigenic expression, were not lysed by A and C. This inability to lyse persistently infected cells was not due to their enhanced membrane resistance or repair, since those cells were lysed efficiently by anti-H-2 antibodies and C. As judged by 50% cytotoxicity end point determinations, uninfected, acutely infected, and persistently infected cells had similar levels of H-2 antigens on their surfaces.

During previous studies of measles virus infection of HeLa cells, we showed that specific anti-viral antibody stripped measles virus antigens off the surfaces

of infected cells (9), making these cells resistant to both lymphocyte-mediated and A- and C-dependent lysis (9, 11). In those studies, the amount of viral antigens expressed on the cell's surface correlated directly with the ability of immune reagents to lyse the cell. Lysis of measles virus-, mumps virus-, and herpes simplex virus-infected cells by specific anti-viral A and C in a homologous system (human cells, A, and C) (9, 10) required the binding of approximately 5–7 $\times$ 10^6 A molecules per infected cell. In the present study, which examined lysis of LCMV-infected cells with a heterologous system (mouse cells, guinea pig A, and C), 5–7 $\times$ 10^5 molecules of A had to bind per infected cell to specifically kill approximately 25–30% of acutely infected cells. In addition to the requirement of A to LCMV and C for immune lysis we noted that late in the acute infection C alone could lyse infected cells. This direct C lysis was not seen with uninfected or persistently infected cells and occurred only at a restricted time period (days 3–7) during acute infection. This A-independent lysis was not associated with the appearance of C3 receptors on infected cells. Both the mechanism of direct C activation and the immunopathological significance of this observation are under investigation. The direct binding to and lysis or inactivation of oncornaviruses (49, 50), Newcastle disease virus (51, 52), and tumors cells (53) by C in the absence of specific antibodies has been reported, and could possibly explain our results.

In contrast to their insensitivity to A-dependent C-mediated lysis, most cells persistently infected with LCMV were susceptible to immune T-lymphocyte-mediated killing. Although 6% or less of these cells had detectable viral antigens on their surfaces by immunofluorescence, significant numbers were immunospecifically killed by sensitized T lymphocytes (46% ^{51}Cr released as compared to 11% control) (Table I). In all eight experiments that tested the ability of sensitized T lymphocytes to kill LCMV acutely and persistently infected target cells, we noted that the greatest cell death occurred 2–4 days after acute infection and that lysis of persistently infected cells was never more than 66% of this maximum amount. Those persistently infected cells which escaped T-lymphocyte killing were infected as judged by both the presence of cytoplasmic LCMV antigens and by the inability of any of these cells to be superinfected with LCMV. In other studies, 100% of the clones isolated from persistently infected cultures contained cells expressing viral antigens (29, 37). Hence, in persistently infected cultures, a significant percentage of infected cells (average, 40% of cells; range, 19–54%) escaped lysis by sensitized T cells under these conditions and nearly all such cells escaped killing by anti-LCMV A and C.

There appears to be two major explanations why a significant number of persistently infected cells were susceptible to T-lymphocyte-mediated lysis but not A-mediated C lysis. First, the T-lymphocyte cytotoxicity assay may be more sensitive than the A and C test. Evidence from several systems support this view (54–56). A second explanation is that T lymphocytes may recognize different sets of antigens or alterations of antigens (22) that are not recognized by anti-viral antibodies. Although our data do not exclude either of these possibilities, the facts that (*a*) DI LCMV turned off S LCMV surface antigen expression and, in turn, the ability of sensitized T lymphocytes to kill virus-infected targets (Table VI) and that (*b*) Fab fragments of guinea pig IgG containing antibodies to LCMV can abrogate both A-initiated C-dependent and T-lymphocyte killing of

LCMV-infected targets (inhibition 70–80%) (M. B. A. Oldstone and T. Tishon, unpublished observations) favor the hypothesis that T lymphocytes and antiviral antibodies recognize similar or related virus determinants but that the T lymphocytotoxicity assay is the more sensitive of the two. Others have shown that for T-lymphocyte killing of LCMV-infected murine targets to occur, both LCMV antigen and H-2 recognition is needed (22, 24). In our experiments, the average concentration of H-2 antigens on surfaces of persistently infected cells approximated that on uninfected or acutely infected cells.

Our results indicated that there was control of viral antigenic expression during the LCMV infection that was a function solely of virus and cell and independent of the immune response. Viral protein synthesis can be inhibited by interferon (57), but LCMV does not induce interferon synthesis in L-929 cells (31, 34, 35). The relatively exclusive release of an interfering component from the persistently infected cells which had little expression of cell surface viral antigens led us to explore the influence of this interfering component on viral antigen synthesis. Results published here and elsewhere (16, 32) clearly indicate that the interfering component released from persistently infected cells is a DI virus: (*a*) it interferes specifically with the synthesis of S LCMV but not with heterologous viruses; (*b*) the interference is not cell species specific; (*c*) it sediments to a density slightly less than S LCMV; (*d*) it has the morphological characteristics of the LCM virion (P. Lampert, personal communication); (*e*) it is inactivated by monospecific antisera to LCMV; (*f*) its UV inactivation kinetics suggest a small nucleic acid target size for interference; (*g*) it is not synthesized unless cells are coinfected with S LCMV. Although no deletion in its RNA has yet been found, a deletion has been reported in the RNA of the DI virus of Pichinde (58), a closely related virus.

Similarly, during the acute LCMV infection in L-929 cells, an interfering component appears just before the shutdown in cell surface antigen synthesis. It has been shown and confirmed that this interfering component resembles the purified DI virus in size, inactivation kinetics, and interference properties (31, 59). Whereas S LCMV synthesis peaked at days 2 and 3 postinfection, the interfering component peaked on days 3 and 4. Antigenic expression on the cell surface was maximum on days 2–4 and then rapidly declined. Hence, there was as association between *de novo* synthesis of an interfering component and a reduction in surface antigen expression, as well as a precipitous reduction in the number of cells scoring as infective centers. The fact that the interfering component when transferred to susceptible cells blocked S LCMV infective center formation, synthesis, and antigen production strongly suggests a cause-effect role of the interfering component in the culture's "turn-off." Others have suggested that a difficult-to-see turbid plaque variant may also be responsible for interference during acute infection (60, 61). This seems unlikely as the turbid plaque variant should produce virus antigens which do not occur in this model. Further, we have been unable to detect turbid plaques in this system, whereas we can detect turbid LCMV plaques on several cell lines with several strains of LCMV (31, 38).

Administration of DI LCMV to cells before or after S LCMV infection curtailed the production of viral antigens and could halt viral antigen synthesis at a time when antigens were easily detected in the cytoplasm but not at the cell

surface. By pretreatment with DI LCMV, cells infected with S LCMV could be rendered resistant to T-lymphocyte-mediated lysis, a very sensitive indicator of surface antigen expression. DI LCMV appeared to block the synthesis of all virion antigens, but whether under certain circumstances it could block preferentially the synthesis of surface but not cytoplasmic antigens is not known but currently is under investigation. The observation that cells contained cytoplasmic but not surface antigens might be explained by a generalized block in viral protein synthesis if there were a more rapid turnover rate of viral antigens on the cell surface than in the cytoplasm. Alternatively, cells could be blocked in viral antigen synthesis at a time early in the infection when cytoplasmic antigens but not surface antigens were present. Support for a generalized reduction in viral protein synthesis comes from current studies using polyacrylamide gel analysis of ^{35}S-methionine pulse-labeled viral proteins demonstrated in LCMV-infected cells. Using 30-min pulses, we noted that the synthesis of the 60,000 dalton structural protein of LCMV (major protein) was undetectable in persistently infected cultures but was readily seen in acutely infected cultures. Superinfection of the persistently infected cultures with S LCMV did not result in detectable synthesis of this structural protein (M. Buchmeier, R. M. Welsh, and M. B. A. Oldstone, unpublished results).

Whether or not DI virus plays a role in the establishment or maintenance of the LCMV-carrier state in the mouse is unclear. Peritoneal cells from persistently infected mice resemble persistently infected cells (which produce DI virus) in culture in that they have reduced expression of surface viral antigens and are resistant to superinfection with LCMV. Previously, Mims and Subrahmanyan (62) showed specific resistance (homologous interference) of peritoneal macrophages from persistently infected mice to LCMV superinfection. They concluded that viral interference may play a role in the carrier state. Our studies confirm and extend their findings and point to the possibility that reduced expression of antigens on the cell's surface may render cells resistant to immunological attack in vivo. Recently we have presented evidence that in vivo synthesis of LCMV antigens in rats can be abrogated by experimental transfer of DI LCMV.[2] The fact that DI virus can be generated in vivo in experimental rabies (63), VSV (63), and reovirus (64) infections suggests that DI virus production may occur during natural infection with LCMV. LCMV-infected mice also have a population of cells totally free of detectable antigens, though the virus is capable of infecting virtually every nucleated cell in the body. This may reflect DI virus-mediated interference, as DI LCMV can block viral antigen synthesis. Data in Table X demonstrate that DI LCMV can interfere with S LCMV synthesis in primary cells taken from normal mice.

Summary

The expression of viral antigens on the surfaces of lymphocytic choriomeningitis virus (LCMV)-infected L-929 cells peaked 2–4 days postinfection and thereafter precipitously declined. Little or no viral antigen was expressed on the

[2] Welsh, R. M., P. W. Lampert, and M. B. A. Oldstone. 1977. Prevention of virus-induced cerebellar disease by defective-interfering lymphocytic choriomeningitis virus. *J. Infect. Dis.* In press.

plasma membrane surfaces of persistently infected cells, but LCMV antigens were clearly present in the cytoplasms of most of those cells. Cells early after acute infection (days 2–4) were lysed by both virus-specific antibody and complement (C) and immune T lymphocytes. To the contrary, antibody and C did not kill persistently infected cells, but T lymphocytes did kill such cells although at a lower efficiency than acutely infected cells. The expression of viral antigens on the surfaces of infected cells was regulated by the virus-cell interaction in the absence of immune reagents and was closely associated with defective interfering (DI) LCMV interference. DI LCMV, per se, blocked the synthesis and cell surface expression of LCMV antigens, and DI LCMV generation immediately preceded a precipitous reduction in cell surface antigenicity during the acute infection. Persistently infected cells produced DI LCMV but no detectable S LCMV. Peritoneal cells isolated from mice persistently infected with LCMV resembled cultured persistently infected cells in their reduced expression of cell surface antigens and their resistance to LCMV superinfection. It is proposed that DI virus-mediated interference with viral protein synthesis may allow cells to escape immune surveillance during persistent infections.

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