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In Vivo Selection of Lymphocyte-Tropic and Macrophage-Tropic Variants of Lymphocytic Choriomeningitis Virus during Persistent Infection

CHWAN-CHUEN KING, RICARDA DE FRIES, SUHAS R. KOLHEKAR, AND RAFI AHMED*

Department of Microbiology and Immunology, University of California, Los Angeles, School of Medicine, Los Angeles, California 90024-1747

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This study demonstrates cell-specific selection of viral variants during persistent lymphocytic choriomeningitis virus infection in its natural host. We have analyzed viral isolates obtained from CD4$^+$ T cells and macrophages of congenitally infected carrier mice and found that three types of variants are present in individual carrier mice: (i) macrophage-tropic, (ii) lymphotropic, and (iii) amphotropic. The majority of the isolates were amphotropic and exhibited enhanced growth in both lymphocytes and macrophages. However, some of the lymphocyte-derived isolates grew well in lymphocytes but poorly in macrophages, and a macrophage-derived isolate replicated well in macrophages but not in lymphocytes. In striking contrast, the original wild-type (wt) Armstrong strain of lymphocytic choriomeningitis virus that was used to initiate the chronic infection and from which the variants are derived grew poorly in both lymphocytes and macrophages. These three types of variants also differed from the parental virus in their ability to establish a chronic infection in immunocompetent hosts. Adult mice infected with the wt Armstrong strain cleared the infection within 2 weeks, whereas adult mice infected with the variants harbored virus for several months. These results suggest that the ability of the variants to persist in adult mice is due to enhanced replication in macrophages and/or lymphocytes. This conclusion is further strengthened by the finding that the variants and the parental wt virus grew equally well in mouse fibroblasts and that the observed growth differences were specific for cells of the immune system.

Previous studies have documented the importance of host tissues in the selection of viral variants during chronic infection (2, 3). We have shown that lymphocytic choriomeningitis virus (LCMV) isolates of different phenotypes predominate in the central nervous system and lymphoid tissue of carrier mice infected at birth with the wild-type (wt) Armstrong strain of LCMV. Most of the central nervous system isolates are similar to the wt Armstrong strain and induce potent virus-specific cytotoxic T-lymphocyte (CTL) responses in adult mice, and the infection is cleared within 2 weeks. In contrast, the majority of the isolates derived from the lymphoid tissue cause chronic infections in adult mice associated with low levels of detectable CTL responses.

The objective of this study was to identify the cell types within the lymphoid tissue in which variants are selected and to characterize LCMV isolates derived from purified cell populations. We have shown that T cells of the helper subset (CD4$^+$) are infected with LCMV during persistent infection in vivo (1). Our findings were subsequently confirmed by Tishon et al (16). In this study, we have obtained LCMV isolates from purified CD4$^+$ T cells and macrophages of carrier mice and studied their biological properties. Our results show that there are three types of variants present in individual carrier mice: (i) macrophage-tropic variants (LCMV isolates that grow well in macrophages but poorly in lymphocytes), (ii) lymphotropic variants (LCMV isolates that replicate well in lymphocytes but not in macrophages), and (iii) amphotropic variants (LCMV isolates that grow well in both macrophages and lymphocytes). These three types of variants are strikingly different from the original wt Armstrong strain, which grows poorly in both macrophages and lymphocytes.

MATERIALS AND METHODS

Mice. BALB/c ByJ mice were purchased from Jackson Laboratory, Bar Harbor, Maine. The congenitally infected LCMV carrier colonies were bred and established at the University of California at Los Angeles. Three-month-old mice at the third generation of a colony congenitally infected with LCMV Armstrong were used for the isolation of viral variants.

Virus. The Armstrong CA 1371 strain of LCMV was used in these studies (3). The virus was triple plaque purified on Vero cells, and stocks were grown in BHK-21 cells. This original laboratory stock of CA 1371 will be referred to as wt Armstrong strain. The carrier colony was originally started by infecting 1-day-old mice with this strain. The macrophage- and lymphocyte-derived isolates of CA 1371 were isolated from highly purified populations of splenic T cells and macrophages obtained from 3-month-old congenitally infected carrier mice (third generation). All viral isolates were plaque purified three times, and virus stocks (grown in BHK cells) at passage 1 or passage 2 levels were used in subsequent experiments. The biological properties of the various isolates are extremely stable in tissue culture, and we have had no reversion of the phenotype during the plaque purification on Vero cells or growth in BHK cells.

Virus titration. Infectious LCMV was quantitated by plaque assay on Vero-cell monolayers as previously described (3).

Infectious center (IC) assay. IC assay was done as previously described (1).

Separation of various spleen and lymph node (LN) cell

* Corresponding author.

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subpopulations. The techniques used for obtaining highly purified populations of various cell types present in spleens and LN have been previously described (1, 11). A combination of positive and negative selection techniques were used, and these procedures yielded cell populations that were ≥95% pure, as determined by fluorescence-activated cell sorter analysis after staining with appropriate antisera.

Infection of macrophages and lymphocytes in vitro with LCMV. Resident peritoneal macrophages from normal 6- to 10-week-old BALB/c ByJ mice were obtained by peritoneal lavage with minimal essential medium (7 ml per mouse) supplemented with 20 U of heparin per ml. Macrophages were washed once with minimal essential medium plus 1% fetal calf serum, counted, and suspended in RPMI 1640 plus 10% fetal calf serum at a concentration of 3×10^5 cells per well in 24-well plates (Costar, Cambridge, Mass.). Cells were allowed to adhere for 3 to 5 h and then washed three times to remove nonadherent cells. Monoclonal antibodies Mac-1 (Hybritech, San Diego, Calif.) and F4-80 were used to confirm the identities of these cells (10). The harvested macrophages were infected with various strains of LCMV at a multiplicity of infection (MOI) of 0.2 PFU per cell in a volume of 0.25 ml. In some experiments, an MOI of 3.0 was used. After 1 h of virus adsorption at 37°C, the inoculum was removed and 2.0 ml of fresh RPMI 1640–10% fetal calf serum medium was added. Supernatant (SN) was harvested from separate wells at various time intervals and stored at −70°C for plaque assay on Vero cells.

The separated T or B lymphocytes were also infected with various strains of LCMV at an MOI of 0.2 PFU per cell (5×10^6 pelleted cells were suspended in less than 0.5 ml of virus). In some experiments, MOIs of 3.0 and 5.0 were used. After 1 h of adsorption, 5×10^5 T or B lymphocytes per well (0.2 ml per well) were cultured in RPMI 1640–10% fetal calf serum with 5×10^{-5} M 2-mercaptoethanol and stimulated with concanavalin A (ConA, Miles-Yeda, Naperville, Ill.) at 2 or 25 μg of lipopolysaccharide (LPS; Difco, Detroit, Mich.) per ml for T and B cells, respectively. Supernatants (SN) and cells were harvested, stored, and titrated as described above.

Immunofluorescence. For immunofluorescence assay, cytocentrifuge preparations of lymphocytes were fixed with acetone and stained with polyclonal anti-LCMV guinea pig serum and then with fluorescein-conjugated rabbit anti-guinea pig immunoglobulin G (Cooper Biomedical, Inc., Malvern, Pa.). LCMV-infected cells stained with normal guinea pig serum and uninfected lymphocytes stained with anti-LCMV guinea pig serum were included as controls.

RESULTS

Isolation of LCMV from lymphocytes and macrophages of carrier mice. Our previous studies have shown that LCMV variants that can cause chronic infection in adult mice are selected in lymphoid tissue of carrier mice infected at birth with the wt Armstrong strain (2, 3). In these earlier studies, LCMV isolates were obtained from a spleen homogenate and thus the identity of the cell types that harbor the variants was not known. To address this question, spleen cells from LCMV carrier mice were fractionated into different cell populations and the number of infected cells was determined by an IC assay. The infection was confined mostly to $CD4^+$ T cells and adherent cells (Table 1). About 1% of the cells in these two subpopulations produced infectious virus. In contrast, there was minimal infection of either $CD8^+$ T cells or B cells (≤0.01% infected cells).

TABLE 1. Identification of cell types infected with LCMV in spleens of carrier mice

Cell population[a]	No. of infected cells[b]/10^6 cells	% Infected cells
Unfractionated spleen cells	5,250	0.52
$CD4^+$ T cells	9,300	0.93
$CD8^+$ T cells	170	0.01
B cells	110	0.01
Adherent cells	12,120	1.21

[a] Spleen cells pooled from six congenitally infected 10-week-old BALB/c mice were used. The indicated cell populations were purified as described in Materials and Methods.

[b] The number of cells producing LCMV was quantitated by an IC assay as described in Materials and Methods, except with the following modifications for the adherent cells: the Vero cells were plated on top of the adherent cells, incubated at 37°C for 4 h to form a monolayer, and then overlaid with agarose containing medium 199.

LCMV isolates were obtained by picking IC formed by $CD4^+$ T cells and adherent cells. These IC were subjected to three rounds of plaque purification to ensure clonality of the virus, and then stocks were grown in BHK cells. Several adherent cell-derived and $CD4^+$ T-cell-derived LCMV isolates were obtained from individual carrier mice, and 10 of these were selected for further study (Table 2). The adherent-cell population consisted predominantly (≥90%) of macrophages, as determined by esterase staining, and therefore, virus obtained from this population will be referred to as macrophage-derived isolates. The $CD4^+$ T-cell-derived virus will be referred to as lymphocyte-derived isolates.

Infection of adult mice with macrophage-derived and lymphocyte-derived LCMV isolates. Six- to eight-week-old BALB/c mice were challenged with the various LCMV isolates, and the level of virus present and the CTL response were checked 8 days postinfection. Mice infected with the macrophage- or lymphocyte-derived isolates contained low levels of LCMV-specific CTL and contained high levels of virus in the serum and spleen (Table 3). High levels of virus were also present in several other tissues (lung, liver, LN, kidney, etc.), and these mice continued to harbor virus for several months (data not shown). In contrast, mice infected with the parental wt Armstrong exhibited a potent LCMV-

TABLE 2. Origin of LCMV isolates used in this study

Virus isolate[a]	Source	
	Spleen cell population[b]	Carrier mouse[c] no.
M2.1A	Adherent cells	1
M5.1A	Adherent cells	1
M10.1A	Adherent cells	2
M17.1B	Adherent cells	3
M21.2B	Adherent cells	3
t1b	$CD4^+$ T cells	1
T5.10A	$CD4^+$ T cells	1
t4b	$CD4^+$ T cells	2
t6b	$CD4^+$ T cells	3
T13.22A	$CD4^+$ T cells	3

[a] Virus isolates were obtained by picking IC formed by $CD4^+$ T cells or adherent cells, and then these IC were subjected to three rounds of plaque purification to ensure clonality of the virus stocks.

[b] Spleen cell populations from individual carrier mice were purified as described in Materials and Methods.

[c] Ten-week-old BALB/c carrier mice; third generation of a congenitally infected carrier colony originally established by injecting 1-day-old mice with the wt Armstrong CA 1371 strain of LCMV.

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TABLE 3. Infection of adult mice with macrophage- and lymphocyte-derived LCMV isolates[a]

Virus isolate	LCMV-specific CTL in spleen[b]				LCMV titer (log_{10} PFU)	
	LCMV infected at E/T ratio of:			Uninfected (E/T ratio, 50:1)	In spleen	Per ml of serum
	50:1	16.6:1	5.5:1			
M2.1A	6	1	0	0	5.47	3.97
M5.1A	15	5	0	0	5.36	4.40
M10.1A	20	8	0	0	4.76	4.49
M17.1B	10	4	1	0	5.11	4.34
M21.2B	3	0	0	0	4.83	4.14
t1b	2	0	0	0	4.40	3.87
T5.10A	8	3	0	0	5.27	4.60
t4b	7	3	0	0	4.20	4.00
t6b	5	0	0	0	4.13	3.65
T13.22A	1	0	0	0	6.06	4.88
Parental wt Armstrong	67	58	28	2	<2.00	<1.60

[a] Adult BALB/c mice were infected intravenously with 2.5×10^5 PFU of the indicated virus, and CTL response and virus titer were checked 8 days after infection. The data shown are the averages of values from two to four mice per group.

[b] Values are the percentage of ^{51}Cr release from BALB C17 (H-2^d) targets.

TABLE 4. Growth phenotype of lymphocyte- and macrophage-derived LCMV isolates[a]

Virus isolate	Isolate titer/wt Armstrong virus titer in:		Growth phenotype[b]
	Lymphocytes	Macrophages	
M2.1A	22.3	10.8	Amphotropic
M5.1A	25.7	13.3	Amphotropic
M10.1A	0.5	10.3	Macrophage-tropic
M17.1B	9.2	9.7	Amphotropic
M21.2B	10.2	16.6	Amphotropic
t1b	15.7	0.2	Lymphotropic
T5.10A	42.0	11.6	Amphotropic
t4b	9.4	0.3	Lymphotropic
t6b	13.1	0.6	Lymphotropic
T13.22A	38.5	11.0	Amphotropic

[a] The indicated viral isolates were checked for their ability to grow in primary cultures of LN cells and PE macrophages. The data are presented as ratios of the titer of isolate and the titer of the parental wt Armstrong virus in LN cells or PE macrophages (at day 3 postinfection). Ratios are averages from three to five experiments.

[b] Viral isolates that grew to substantially higher titers (approximately 10-fold greater) in both lymphocytes and macrophages than the parental wt Armstrong strain did were classified as amphotropic. Those isolates that grew well in only macrophages of lymphocytes were classified as macrophage-tropic or lymphotropic, respectively.

specific CTL response, and the virus was cleared within 2 weeks (Table 3). These results confirm our earlier findings and extend these observations by showing that LCMV variants with the ability to cause chronic infections in adult mice are selected in both macrophages and $CD4^+$ T cells (2, 3).

Growth phenotype of LCMV isolates. To further characterize the variants and to determine the underlying basis for their ability to persist in adult mice, the various viral isolates were tested for their ability to grow in primary lymphocytes and macrophages. Lymphocytes were obtained from LN of adult mice, and resident peritoneal-exudate (PE) adherent cells were used as a source of macrophages. Primary cultures of LN cells and PE macrophages were infected with the parental virus and the various LCMV isolates, and the amount of virus released in the SN was quantitated by a plaque assay. These in vitro studies revealed four patterns of growth, and data from isolates representing each pattern are shown in Fig. 1. The original wt Armstrong grows poorly in both LN cells and macrophages. In contrast, the isolate T5-10A exhibited good growth in both LN cells and macrophages: ≈100-fold-higher level in LN cells and ≈20-fold-higher level in macrophages compared with levels of the parental virus. The majority (6 of 10) of the LCMV variants analyzed in this study exhibited this amphotropic growth pattern (Table 4). Isolate M10-1A was macrophage-tropic in that it showed enhanced replication in macrophages (≥25-fold higher) but grew as inefficiently as the wt Armstrong in LN cells. The fourth pattern of growth (i.e., lymphotropic) is represented by the isolate t1b; this variant showed good growth in LN cells (≈100-fold higher) but grew to even lower titers than the parental virus did in macrophages. These results show that there are three types of variants present in individual carrier mice: (i) macrophage-tropic, (ii) lymphotropic, and (iii) amphotropic variants.

Viral growth in LN cell cultures was seen only when the

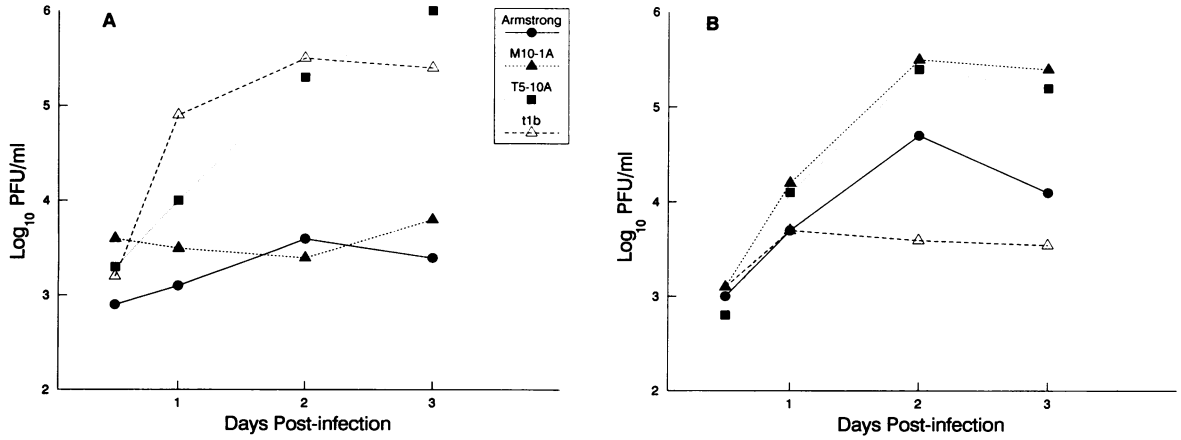

FIG. 1. Growth of LCMV variants in primary lymphocytes and macrophages. Primary cultures of LN cells (A) or resident PE macrophages (B) were infected with the indicated LCMV isolate at an MOI of 0.2, and the amount of infectious virus released in the SN was quantitated by a plaque assay. The LN cells were cultured in the presence of 25% ConA SN.

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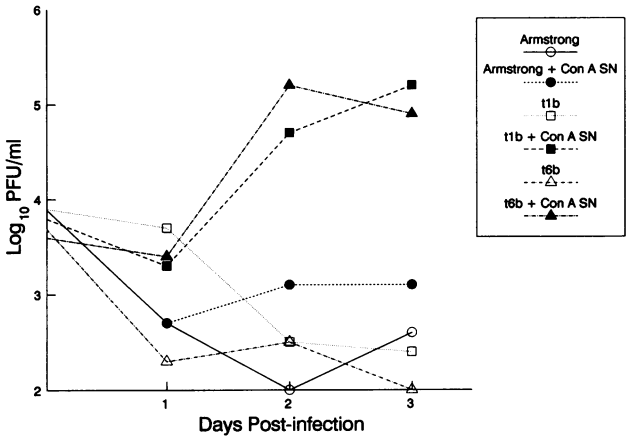

FIG. 2. Growth of LCMV isolates in LN cells with or without stimulation with ConA SN.

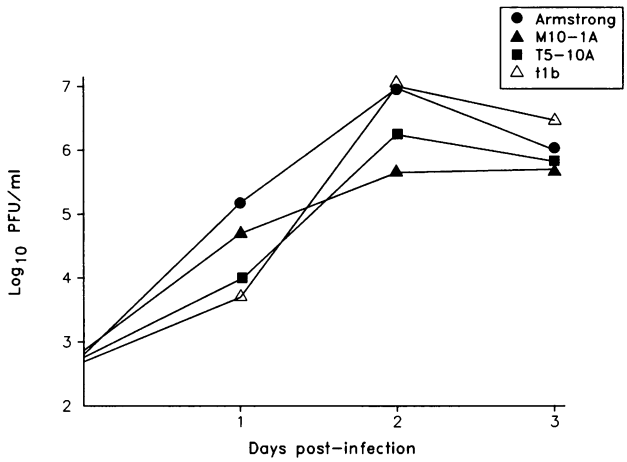

FIG. 3. Growth of LCMV variants in mouse fibroblast cells. BALB Cl7 cells, a continuous fibroblast cell line derived from BALB/c mice, were infected with the indicated LCMV isolate at an MOI of 0.2, and the amount of infectious virus released in the SN was quantitated by a plaque assay.

lymphocytes were stimulated with mitogen or ConA SN. Neither the parental Armstrong strain nor any of the amphotropic or lymphotropic variants showed any growth in resting lymphocytes. However, following stimulation of lymphocytes with ConA SN, there was an ≈50- to 100-fold increase in titers of the variants. The growth curves of the parental wt virus and the variants t1b and t6b in resting and activated LN cells are shown in Fig. 2. As can be seen from these data, the parental Armstrong showed minimal to no growth in resting or activated LN cells, whereas t1b and t6b show enhanced replication in the activated cultures.

The various viral isolates were also tested for their ability to grow in primary and continuous fibroblast cell lines. No major or consistent differences were found among the isolates and the parental virus, and all isolates tested, including the parental wt Armstrong virus, grew well in mouse fibroblast cells. The data from one such experiment showing the growth of M10-1A (macrophage-tropic), t1b (lymphotropic), T5-10A (amphotropic), and the parental wt Armstrong virus in BALB Cl7 cells, a continuous mouse fibroblast cell line, are presented in Fig. 3. Similar results were obtained with primary cultures of mouse embryo fibroblasts (data not shown). Thus, the observed growth differences between the variants and the parental virus (Fig. 1 and Table 4) are specific for cells of the immune system and are not seen in fibroblasts.

Characterization of cell types infected by lymphotropic isolates of LCMV. To identify the cell types infected by the lymphotropic isolates of LCMV, different subpopulations (macrophages, B cells, and $CD4^+$ and $CD8^+$ T cells) were depleted and the ability of LCMV variants to grow in these depleted LN cultures was determined. The data for one of these isolates, t1b, are shown in Fig. 4. Removal of macrophages, either by treatment with anti-Mac-1 monoclonal antibody plus the complement or by passing LN cells through a G-10 column, had no effect on the titer of t1b. These results show that replication in macrophages did not contribute to the enhanced yield of virus seen in LN cultures. In contrast, depletion of T cells or of $CD4^+$ T cells caused a substantial decrease in the level of virus production, indicating that $CD4^+$ T cells were required for optimal viral growth. Depletion of $CD8^+$ T cells had no effect, showing that this T-cell subset was not susceptible to infection by the t1b variant. Surprisingly, treatment of LN cells by anti-J11d monoclonal antibody plus the complement (a marker for B cells) reduced the viral yield, suggesting that growth in B cells was also contributing to the total viral yield seen in LN cultures.

To confirm the results shown in Fig. 4, growth of t1b was checked in highly purified populations of T and B cells. T and B cells were obtained from LN as described in Materials and Methods (>95% pure populations). Following infection with virus, the T-cell cultures were stimulated with ConA and the B-cell cultures were stimulated with LPS. The LCMV variant t1b grew equally well in both cell populations (Fig. 5), confirming the results obtained by the negative-depletion experiments shown in Fig. 4. Similar results were obtained with t6b (data not shown).

The identity of the cell types infected by t1b was further checked by an IC assay. In T-cell cultures stimulated with ConA, the infected cells were typed as T cells, whereas in B-cell cultures stimulated with LPS, the IC were not de-

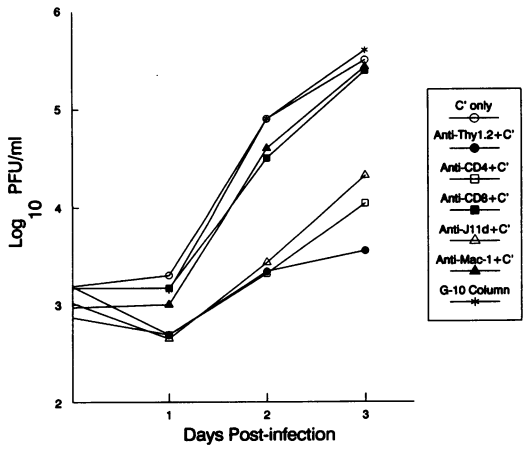

FIG. 4. Characterization of cell types infected with lymphotropic isolate of LCMV. Various LN subpopulations (CD4 and CD8 cells, B cells and macrophages) were depleted, and the ability of LCMV isolate t1b to grow in these depleted LN cultures was determined. The cells were cultured in the presence of 25% ConA SN. C', Complement.

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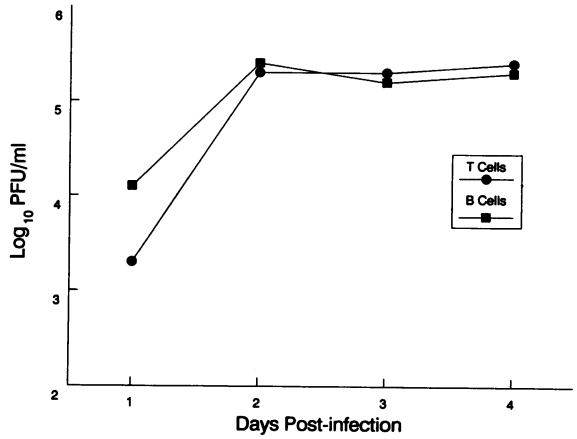

FIG. 5. Growth of LCMV isolate tlb in primary cultures of purified B cells and T cells. The B cells were cultured in the presence of 25 μg of LPS per ml, and the T cells were cultured with 2 μg of ConA per ml.

creased by anti-Thy antibody plus complement treatment but were reduced by anti-J11d antibody plus complement (i.e., were typed as B cells) (Table 5). In experiments using whole LN cells, the cell population infected depended upon the mitogen used. When the B-cell mitogen LPS was used, the infection was almost exclusively of B cells, whereas when the T-cell mitogen ConA was employed, the infection was confined predominantly to T cells.

Interestingly, in all of these experiments, only a small fraction (≈1%) of the lymphocytes scored as IC. In most experiments, a MOI of 0.2 was used. However, even in experiments using an MOI of 5.0, the percentage of cells scoring as IC was only ≈1%. In a time course experiment, the numbers of IC were checked at days 2, 3, and 4 postinfection. The percentage of infected cells varied between 0.5 to 1.3%, similar to the data shown in Table 5. The

TABLE 5. Quantitation and identification of cell types infected with LCMV isolate tlb by IC assay

LN cells infected[a]	Mitogen	Depletion agent[b]	No. of IC/10^6 cells	% Infected cells
T cell enriched	ConA	Complement only	11,000	1.1
		Anti-Thy	1,400	
		Anti-J11d	6,000	
B cell enriched	LPS	Complement only	12,500	1.2
		Anti-Thy	10,500	
		Anti-J11d	3,500	
Lymphocytes	ConA	Complement only	11,500	1.1
		Anti-Thy	2,500	
		Anti-J11d	6,500	
Lymphocytes	LPS	Complement only	8,000	0.8
		Anti-Thy	11,000	
		Anti-J11d	2,000	

[a] LN cell populations were infected with LCMV tlb 4 days prior to IC assay. T-cell-enriched populations were cultured with the addition of 2 μg of ConA per ml, and B-cell-enriched populations were cultured with 25 μg of LPS per ml.

[b] Prior to being added to a susceptible cell monolayer, infected cells were depleted with antibody and complement to determine whether they were T cells (anti-Thy plus complement) or B cells (anti-J11d plus complement).

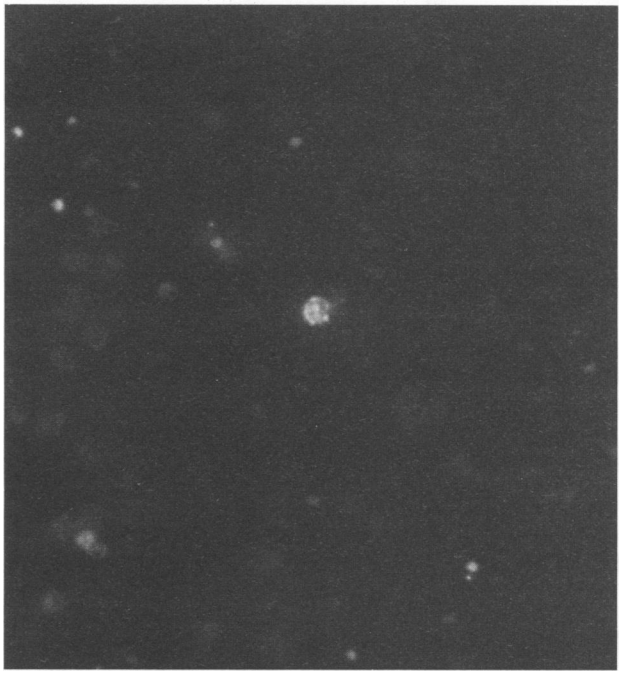

FIG. 6. Immunofluorescence staining demonstrating viral antigen in purified T cells infected with LCMV isolate tlb. T cells were purified from LN as described in Materials and Methods and infected with tlb at an MOI of 3.0. The cells were fixed and stained for viral antigen at 3 days postinfection.

number of infected cells was also checked by staining cells with LCMV-specific antisera. The fraction of virally infected cells as determined by immunofluorescence was also very low (≈1%). Immunofluorescent staining demonstrating viral antigen in a purified population of T cells is shown in Fig. 6.

DISCUSSION

This study documents cell-specific selection of viral variants during persistent infection in the natural host. We have analyzed LCMV isolates derived from purified $CD4^+$ T cells and macrophages of congenitally infected carrier mice and shown that three types of variants are present in individual carrier mice: (i) macrophage-tropic, (ii) lymphotropic, and (iii) amphotropic (i.e., enhanced growth in both lymphocytes and macrophages) variants. In contrast, the original wt Armstrong strain that was used to initiate the chronic infection and from which the variants are derived grows poorly in both lymphocytes and macrophages.

The emergence of cell-specific viral variants can be explained by the fact that when a viral infection occurs in the whole animal, the various organs and cell types present in the body provide a rich milieu for the selection of viral variants. During long-term persistence in carrier mice with continuous virus replication, and given the relatively high mutation rate of RNA viruses (9, 15), LCMV variants that have a growth advantage in certain cell types are likely to emerge. The present study demonstrates that lymphocytes favor the selection of lymphotropic variants, macrophages favor the selection of macrophage-tropic variants, and both of these two types of cells provide additional selective advantage for the emergence of amphotropic variants. It is possible that lymphotropic as well as macrophage-tropic

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variants derived from the parental wt Armstrong strain are intermediate products during the sequential adaptation of viral variants and that the final outcome of this evolution is to select amphotropic variants. Our results indicate a possible mechanism by which viral variants emerge in nature and apply, in particular, to viruses causing chronic infection (4). This study may provide a framework for understanding the evolution of human immunodeficiency virus; human immunodeficiency virus is known to persist in both T cells and monocytes, and lymphocyte-specific and macrophage-specific variants of the virus have been isolated from infected individuals (5, 6, 8, 12).

Infection of T cells in vivo during chronic LCMV infection has been documented, but it has been difficult to demonstrate infection of lymphocytes in vitro (1, 7, 13, 14, 16). Previous attempts to infect lymphocytes in vitro with the available LCMV strains have been unsuccessful (13). Our results suggest that these earlier experiments were unsuccessful because the appropriate LCMV isolates were not used. As shown in this paper, the wt Armstrong strain grows poorly in lymphocytes and only LCMV isolates derived from the lymphoid system were able to infect lymphocytes in vitro.

In agreement with our studies showing in vivo infection of $CD4^+$ T cells, the T cells infected in vitro were also of the helper-inducer subset, and there was minimal to no infection of $CD8^+$ T cells. Interestingly, only a small proportion (1%) of $CD4^+$ T cells were infected, even when an MOI of 5 was used. These results suggest that only a particular type (subset) of $CD4^+$ T cells is susceptible to infection by LCMV. It should be pointed out that the percentage of T cells infected in carrier mice is also about 1 to 5% (1, 7, 14, 16). A surprising finding was the result showing in vitro infection of B cells (approximately 1%) by the lymphotropic isolates. This observation differs from the in vivo findings in which the level of infected B cells is much lower (<0.01%). This may reflect differences in the state of activation of the various lymphocytes in vitro and in vivo. In any case, our results clearly demonstrate that LCMV variants capable of infecting both T (CD4) and B cells are selected in lymphocytes of carrier mice.

All of the macrophage- and lymphocyte-derived isolates described in this study were able to persist in adult immunocompetent mice for several months. In contrast, adult mice infected with the parental Armstrong strain cleared the infection within 2 weeks. On the basis of the results presented in this study, it is likely that better replication in lymphocytes and/or macrophages is the underlying cause of this chronic infection. It should be noted that the variants and wt virus grew equally well in primary or continuous fibroblast cells and that the observed growth differences were specific for lymphocytes and macrophages. Experiments are currently in progress to understand how these variants grow more efficiently in lymphoid tissue and to determine the step at which growth of the parental virus is restricted.

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