

**Infectious Lymphocytes  
in Mice Persistently Infected with Lymphocytic  
Choriomeningitis Virus**

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During persistent infection of mice with the lymphocytic choriomeningitis (LCM) virus approximately 3% of leukocytes were found to contain viral antigen and to produce infectious virus. Morphologically, infected cells were shown not to be lymphoblasts and their numbers were not reduced by removal of mononuclear phagocytes. We conclude that in LCM virus carrier mice true lymphocytes participate in the infectious process.

The realization that chronic illnesses of animals and man may be caused by viruses has made the study of persistent virus infections an important task for virologists and immunologists alike, and much work is at present directed at analysing the mechanisms which allow infectious agent and host to co-exist for prolonged periods of time. Of the models employed, the lymphocytic choriomeningitis (LCM) virus carrier mouse has been most extensively studied. Yet gaps of knowledge still prevent a full understanding of the relationship between LCM virus and its natural host. Of the open questions we have tried to answer one we considered most important, namely, whether lymphocytes participate in the infectious process; our results indicate that they do.

Most experiments were done with the WE strain of LCM virus<sup>1</sup>. Infectious virus (PFU) and virus plaque-forming cells (V-PFC) were titrated on L cell monolayer cultures using an agarose-containing overlay medium<sup>2</sup>. Numbers of V-PFC were also determined in mice<sup>3</sup>, the latter being approximately 5 times more susceptible than L cell cultures (unpublished). Colony-bred NMRI mice were employed. Persistent infection was established by infecting mice when less than 24 h old<sup>4</sup>. One naturally infected wild carrier house mouse trapped in North Germany was also investigated. Anti-LCM virus antiserum directed against all immunogenic components of the virus was prepared in rabbits<sup>5</sup>. Goat

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anti-rabbit Ig antiserum labelled with fluorescein isothiocyanate was purchased. Blood cells were separated into leukocyte-rich and erythrocyte-rich fractions by spontaneous sedimentation through a solution of dextran and sodium metrizoate in water<sup>6</sup>. Alternatively, cells from blood or lymphoid organs were fractionated by Ficoll-sodium metrizoate (Lymphoprep) centrifugation<sup>7</sup>. The fractions thus obtained were (from top) (I) cell-free plasma; (II) interface consisting predominantly of thrombocytes, lymphocytes, and monocytes (lympho-monocytes); (III) Lymphoprep consisting predominantly of lympho-monocytes and granulocytes; (IV) pellet consisting predominantly of erythrocytes, granulocytes, lympho-monocytes. Cells of interface (fraction II) were centrifuged again at 100 g, which led to the formation of two subfractions called II a and II b consisting predominantly of thrombocytes and lympho-monocytes, respectively. For the removal of phagocytic elements suspended cells were incubated with carbonyl iron. Non-phagocytic cells were decanted, while iron-carrying elements were held back with a magnet<sup>8</sup>. Of the cells which had thus been freed of phagocytes and were subsequently separated as subfraction II b as described, more than 99% had the morphological characteristics of lymphocytes.

For microscopic visualization of cells, cytocentrifuge preparations were stained with May-Grünwald-Giemsa dye. Cells of the mononuclear phagocytic system were identified histochemically<sup>9</sup>. Viral antigens inside cells and on cell surfaces were localized by immunofluorescence techniques using standard procedures<sup>10, 11</sup>.

When blood from carrier mice was separated by centrifugation and PFU and V-PFC, respectively,

Table I. Concentrations of virus plaque-forming cells in leukocyte- or erythrocyte-rich fractions of blood from LCM virus carrier mice.

Fraction *	Number per ml			% V-PFC
	Erythrocytes	Leukocytes	V-PFC	
Leukocytes	$9.5 \times 10^5$ **	$8.0 \times 10^5$	$1.53 \times 10^4$	0.9
Erythrocytes	$1.7 \times 10^9$	$1.0 \times 10^6$	$2.50 \times 10^4$	≈ 0.001

\* Blood was separated into an upper leukocyte-rich and a lower erythrocyte-rich fraction by spontaneous sedimentation of cells through a solution of 6% dextran and 32.8% sodium metrizoate in water. Fractions were collected, washed by centrifugation, and resuspended to be titrated in L cell monolayer cultures.

\*\* All values are means from individual determinations of three mice.



Table II. Effect on number of virus plaque-forming cells of removal of phagocytic blood cells of carrier mice.

Starting material	V-PFC per ml	
	Treated samples *	Control samples
Whole blood cells	$1.2 \times 10^4$	$1.4 \times 10^4$
Lympho-monocytes **	$2.4 \times 10^4$	$2.9 \times 10^4$

\* Phagocytic cells removed by iron treatment.

\*\* Blood cells were fractionated by Lymphoprep centrifugation and fraction II (interface) was further separated by centrifugation at  $100 \times g$  into subfraction IIa, consisting predominantly of thrombocytes (discarded), and subfraction IIb, consisting predominantly of lympho-monocytes.

were determined in plasma and cells, in repeated experiments always more than 90% of the total infectivity was found to be associated with the cells. Next, cells were separated into leukocyte- and erythrocyte-rich fractions. The results (Table I) show that infectivity was predominantly associated with the leukocytes. Further fractionation was done by Lymphoprep centrifugation. V-PFC were found to be concentrated in subfraction IIb which contained predominantly lymphocytes and monocytes, the latter comprising approximately 10% of the mononuclear cells. Removal by iron treatment of more than 95% of the monocytes from subfraction IIb did not reduce the numbers of V-PFC in comparison with untreated samples of the same cell suspension (Table II), which led us to conclude that the infectious cells in the blood of LCM virus carrier mice were lymphocytes.

The blood of one naturally infected carrier house mouse was also investigated. As in NMRI mice, infectivity was predominantly cell associated and V-PFC were enriched in fraction IIb. Obviously, infection of lymphocytes in LCM virus carrier mice is a general phenomenon.

Cultivated white blood cells could not be infected by LCM virus unless they had been stimulated with phytohemagglutinin<sup>12</sup>, suggesting the possibility that the V-PFC in the blood of carrier mice were lymphoblasts. However, of 7,000 lymphocytes examined in smears of several preparations, lymphoblasts or related cells never exceeded 0.01%. These preparations always contained at least 10 times more V-PFC. The numbers of lymphocytes scoring as V-PFC on L cell monolayers varied between 0.2 and 1% in different experiments with an overall mean of roughly 0.5%. When V-PFC were quanti-

tated in the more susceptible mouse, the mean was found to be approximately 3%, and approximately the same proportion of purified lymphocytes contained LCM virus-specific antigen either inside the cells (Fig. 1) or on their surface.

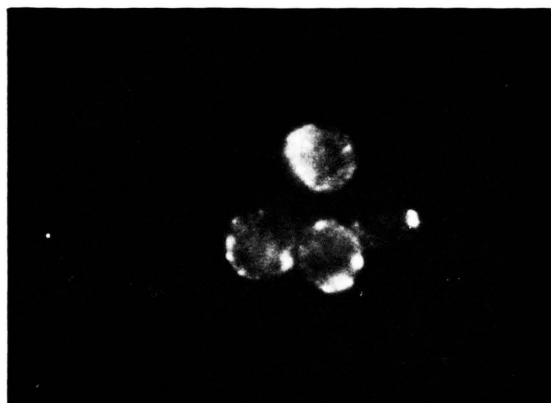


Fig. 1. Lymphocytes, purified from blood of LCM virus carrier mice. Fixed cells were stained with rabbit anti-LCM virus antiserum in combination with fluorescein isothiocyanate-labelled goat anti-rabbit Ig antiserum and were examined with a fluorescence microscope.

Mutatis mutandis, essentially the same results were obtained with purified lymphocytes from spleen and thymus, and we conclude that approximately 3% of the lymphocytes either residing in the central lymphoid organs or circulating in the blood of LCM virus carrier mice are infected with the virus and are infectious.

In contrast to LCM virus carrier mice, the LCM virus has not been demonstrated to replicate in lymphocytes during acute infection of adult mice. Infectious virus was not produced by these cells and viral antigens were not detected by immunofluorescence methodology (Lehmann-Grube and Slenczka, unpublished).

Inability of the persistently infected mouse to eliminate the virus is regarded by most as an example of virus-specific immunological tolerance and has been explained by clonal inactivation<sup>13</sup>. It is tempting to speculate that V-PFC in LCM virus carrier mice are clonally expanded LCM virus-reactive lymphocytes which have lost their immunological function against the viral antigens they now themselves produce.

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