

Low-Pathogenicity Variant of Lymphocytic Choriomeningitis Virus

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A noncytopathic virus has been isolated from a line of strain L mouse fibroblasts infected with lymphocytic choriomeningitis (LCM). This virus has low pathogenicity for mice and renders them immune to intracerebral challenge with virulent LCM virus.

A variant of lymphocytic choriomeningitis virus (LCM) of low pathogenicity (LPV) was isolated from a line of mouse strain L cells persistently infected with the UBC variant (2) of the WE strain of LCM. The variant was not found in persistently infected L cell lines established with the turbid-plaque-type (viscerotropic) LCM strains. It was found only in lines infected with the lytic plaque type or the neurotropic wild virus. An apparently identical LPV isolate has also been obtained from a persistently infected baby hamster kidney (BHK) 21 cells under similar conditions. It appeared to be derived from the clear-plaque-type (3) (neurotropic) variant after subculture of the infected cells through many generations (ranging from 5 to 40 for different isolates) cultivated as described below.

Cultures persistently infected with LPV were passed by trypsinization and inoculation of 10^5 cells into 8 ml of medium (Eagle medium with 10% fetal bovine serum) in a 6-cm plastic petri dish every third or fourth day of CO_2 incubation at 35 C. Passage at lower cell densities from cultures harvested when growth was still only sparse resulted in progressive diminution of specific immunofluorescence and no detectable virus. Under these conditions (10^5 cells/dish), 20 to 40% of the cells were fluorescent positive after staining for LCM antigen. Positive cells showed typical punctate cytoplasmic fluorescence.

Cultures persistently infected with LPV were subjected to a plaque assay sensitive to other LCM strains. No virus could be detected, but animal and tissue culture inoculations demonstrated the presence of a transmissible replicating agent. A pool of medium (LPV fluid) from a persistently infected culture was made, and a 0.22- μ m filtrate was prepared. The virus was detected by its ability to induce fluorescence in

normal L cell cultures after inoculation of 1 ml of filtered medium onto a 6-cm monolayer of freshly subcultured normal L cells; 8 ml of medium was added after 1 h of adsorption. Fluorescence similar to that of the parent culture was only detectable by 4 days and was comparable in extent to the parent culture after 12 days and two transfers of the cells—a period three or four times longer than would be expected after inoculation of terminal dilution of wild LCM virus of either plaque type. BHK cell plaque assay of the filtered LPV fluid produced no plaques at any dilution from undiluted to 10^{-7} stained on any day between 4 and 12 h after inoculation. Two more serial passages of filtered (0.22 μ m) fluid were made; the third-passage material caused the same specific immunofluorescence and immunization of mice.

Inoculation of mice produced the results shown in Table 1. When animals inoculated with LPV were challenged with virulent LCM (UBC strain) 1 to 4 days after subcutaneous (s.c.) inoculation, they all died a day or two earlier than controls challenged the same day. It was concluded that the s.c. inoculation gave sensitization (5) to early intracerebral (i.c.) challenge with LCM virus and partial active immunity to late i.c. challenge.

Virus was present in brain tissue at 4 days after i.c. inoculation of newborn mice with LPV fluid, as revealed by its ability to immunize adult mice (i.c.) against i.c. LCM challenge. Titration of the 4-day brain suspension by this method gave a titer of 10^2 mean infective doses per g in adult mice. Similar titration of the LPV fluid in newborn mice (30 mice/dilution) gave a titer of 50 by mortality and 2.5×10^3 mean infective doses per g in adult mice by i.c. inoculation (no deaths) followed by challenge with virulent LCM virus. Attempts to isolate virulent

TABLE 1. Results of mouse inoculation with LPV fluid.

Inoculum	Route	No. of mice	Age	Deaths	Deaths (%)	LCM challenge deaths ^a	Endotoxin challenge deaths ^b
LPV	s.c.	20	Adult	0	0	2/5	ND ^c
LPV	i.c.	28	Adult	2	7	0/26	1/5
LPV	FP ^d	20	Adult	FP ^d	0	3/20	ND
LPV	i.c.	32	Newborn	5 ^e	15.6	1/9	ND
LPV	i.v. ^f	15	Adult	0	0	15/15	ND
Clear-plaque-type LCM	i.c.	20	Adult	20	100		100%
Clear-plaque-type LCM	i.c.	20	Newborn	20	100		ND

^a Ten thousand mean lethal doses of clear-plaque type given i.c. on day 14 postinfection.

^b *Escherichia coli* endotoxin (100 µg; Difco) administered intraperitoneally on day 7 postinfection (with a different group of mice), lethal for mice incubating wild LCM strains (1).

^c ND, Not done.

^d FP, Footpad response (not deaths).

^e Ten survivors showed no virus carried in blood, brain, spleen, or liver 25 days postinfection.

^f i.v., Intravenous.

virus from the LPV pool by rapid blind i.c. passage (every 3 or 4 days) in young adult mice failed; all evidence of the virus disappeared after the second passage. There was no apparent tendency toward back mutation of the LPV mutant to the parent type, and multiplication in adult mouse brain was minimal. Mice nursing newborns which were inoculated at birth with LPV did not become immune to LCM virus challenge, as did those nursing newborns inoculated with the parent virus. Some of the LPV fluid was ultraviolet (UV) treated (2 min at 22 cm from a GE G30T8 30-W UV lamp at a depth of 1.06 mm)—a dose sufficient to lower LCM titer by 6 log₁₀ units. The UV-treated preparation had no ability to immunize.

The impression that LPV may be slow growing is supported by the fact that it does not develop plaques on BHK 21 cells in 7 days, as do all other LCM strains. Moreover, it takes longer than the parent strain to induce specific immune fluorescence in L cell cultures; it grows in vitro and in vivo only to low titers, and it fails to induce persistent infection in the newborn mouse. It is possible that the undetected presence of this variant causing cyclical transient infection (J. Hotchin, *Nature* [London], in press) will explain the behavior of persistently infected LCM cultures described by other workers (4, 6).

LPV is of low pathogenicity; undiluted tissue culture fluid caused only 7% mortality after i.c. inoculation of adult mice and 15% mortality in newborn mice, compared with 100% mortality in both for the parent virus. Lower i.c. doses failed to cause any mortality in adults or newborns. The lack of deaths or immunity after

intravenous (i.v.) inoculation of the LPV virus is at present unexplained. In limited studies the variant has shown no tendency to revert to the original, more virulent, form. A test of the ability of three separate s.c. vaccinations of mice with 0.1 ml of LPV fluid, followed by i.c. challenge as described above, gave survival values of 5/79 (6.3%), 38/80 (48%), and 55/80 (69%) after one, two, or three vaccinations, respectively.

Since there are many parallels between LCM and the other arenaviruses, it is possible that wild strains of these may also produce slow-growing, less pathogenic variants with vaccine potential.

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