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Characterization of a Nonvirulent Variant of Lymphocytic Choriomeningitis Virus

Brief Report

By

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With 1 Figure

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Summary

A cold-adapted, nonvirulent variant of the Armstrong strain of lymphocytic choriomeningitis virus was isolated from infected L929 cells maintained at 25° C. This variant, designated P17, was capable of replicating in the central nervous system of mice without causing disease and conferring immunity to back challenge with the parental strain.

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Lymphocytic choriomeningitis virus (LCMV) is the prototypic Arenavirus (16). As a group, the *Arenaviridae* have a single-stranded, negative-sense RNA genome with a molecular weight between $4—5 \times 10^6$ (1). The genome has two segments, large (L) and small (S) and the viruses can genetically reassort (1). Recently (11), it was suggested that the L RNA gene products determine the plaque phenotype of LCMV while the S RNA gene products determine pathogenic potential. LCMV has been studied for many years as a model of acute viral disease and viral persistence (7, 10, 12, 13, 17). Adult mice inoculated intracerebrally (i.c.) with LCMV die of acute aseptic meningitis, while newborn mice survive an indentical inoculum. Infection of the newborn results in a persistent infection that lasts the life of the animal and appears to be due to tolerance of the cell mediated immune system (2). In an attempt to better understand the pathogenic potential of LCMV, we describe in this report the isolation and partial characterization of a variant of LCMV that is nonvirulent for adult mice.

The Armstrong strain of LCMV was obtained from R. M. Welsh, jr., Scripps Clinic and Research Foundation, La Jolla, CA. Virus was plaque assayed on MDCK cells (C. J. Pfau, Rensselaer Polytechnic Institute, Troy, NY) using a

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0.5 percent agarose overlay as described by Dutko and Pfau (3). The parent LCMV was neurovirulent having an LD_{50} titer of $10^{-5.50}$/0.03 ml by i.c. inoculation into 4—6 week old Swiss-Webster (SW) mice. In preliminary experiments, we noted that L929 cells (American Type Culture Collection, Rockville, MD) infected with LCMV and incubated at 25° C released virus continuously for 16—18 days until the monolayers began to degenerate. In an attempt to isolate variant viruses, we serially passaged LCMV in L929 cells at 25° C every 14 days. Each passage was tested for virulence by i.c. inoculation into SW mice. No change in the virulence of the virus propagated at 25° C was noted until passage 15. This passage showed reduced virulence ($LD_{50} = 10^{-4.10}$/0.03 ml) compared to the parent virus. Passage 16 showed a significant reduction in neurovirulence ($LD_{50} = 10^{-1.90}$/0.03 ml), and at passage 17 we were unable to establish an LD_{50} as no animals died following injection of up to 2000 PFU of the virus. When we examined the efficiency of plaquing (EOP) at 25°/35° C for each of these passages we found that the ability of passage 15 to form plaques at these two temperatures was essentially equivalent (EOP = 0.90) and virtually indentical to the parent virus (EOP = 0.95). Passage 16 had an EOP of 0.32, and passage 17 (P17) had an EOP = 0.15, indicating that concomitant with its loss of virulence it had lost most but not all of its ability to form plaques at 35° C. P17 was then plaque purified by two passages in MDCK cells. These plaques were primarily of the turbid type (5, 6).

Preliminary studies showed that mice were unaffected following i.c. inoculation with 1000 PFU of P17 which represented approximately 500 LD_{50} of the parental LCMV. A group of mice was inoculated i.c. with 1000 PFU of P17. At various

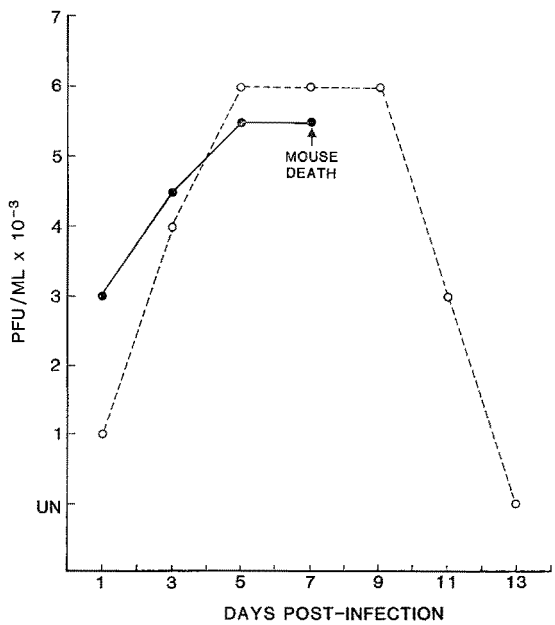

Fig. 1. Replication of P17 (o———o) and Armstrong LCMV (•———•) in mouse brain assayed at 25° and 35° C, respectively, on MDCK cells. *UN* undetectable

days post-infection, animals were sacrificed and one-half of the brain was homogenized (20 percent w/v in Earle's balanced salt solution with 1 percent fetal bovine serum) and assayed at 25° C for infectious virus. The remaining brain half was fixed with formalin and examined for histologic evidence of infection. Fig. 1 compares the replication of P17 in mouse brain assayed at 25° C to the kinetics of virus replication in a group of mice infected with 100 LD_{50} of parental LCMV. P17 was recoverable at 1 day post-infection, the virus titer peaked at 5 days post-infection, and had returned to undetectable levels by day 13. After day 11, virus could not be recovered by homogenization or co-cultivation of brain tissue with L929 or MDCK cells at either 25° or 35° C. Although we clearly demonstrated replication of P17, we were unable to detect any histological changes associated with virus replication. This was in sharp contrast to infection with parental LCMV which showed evidence of meningitis as early as day 4 post-infection. In addition to an unremarkable histological picture, animals infected with P17 did not synthesize detectable levels of neutralizing antibody, although low levels of complement fixing antibody were detectable by day 7 post-infection and the animals were resistant to backchallenge with 100 LD_{50} (216 PFU) of parental LCMV (Table 1). Histologically the back challenged mice showed a mild meningitis by 6 days post-infection that resolved by 14 days post-infection. We could isolate infectious virus for only three days post challenge in these mice.

Table 1. *Effects of LCMV P17 replication in mice*

Days post-infection[a]	LCMV P17[b] present in brain	Complement-fixing[c] antibody titer	Percent surviving challenge[d]
0	+	<1:2	0± 0
1	+	<1:2	0± 0
2	+	<1:2	0± 0
3	+	<1:2	0± 0
4	+	<1:2	0± 0
5	+	1:2	0± 0
7	+	1:4	30±20
9	+	1:4	40±10
11	+	1:4	50±10
13	−	1:8	100± 0
18	−	1:8	100± 0
21	−	1:4	100± 0

[a] 1000 PFU P17 intracerebrally
[b] Titrated in MDCK cells at 25° C
[c] Titers of sera pooled from ten mice determined using the method of KENT and FIFE (9)
[d] 100 LD_{50} parental LCMV intracerebrally

To determine if interference phenomena were responsible for the lack of virulence in P17, we assayed both animal tissues and cell culture fluids for interferon and defective-interfering (DI) particles using published procedures (4, 14). Interferon could be detected in the medium from L929 cultures infected with parental LCMV maintained at 35° C. Interferon was not detected in the

medium of L929 cultures infected with either parental LCMV or P17 incubated at 25° C. In addition, no interferon was detected in brain homogenates of animals infected i.c. with either virus. Since SW mice do not produce high levels of interferon (8, 15), this result was not surprising. To eliminate DI particles in P17, we made two high dilution passages in L929 cells before plaque purification. We checked this pool for the presence of large numbers of DI particles in the medium by plaque assay since DI particles in high concentration cause a decrease in LCMV plaques at low dilutions (14, 18). This could not be demonstrated with P17.

The stability of P17 was examined by making serial brain passages. Briefly, mice were inoculated i.c. with 1000 PFU of P17. At 3 days post-infection, brain homogenates were prepared and were assayed for plaque formation at both 25° and 35° C. After this titration, 1000 PFU was again inoculated i.c. into adult mice. This was repeated three times. There was no increase in virulence with passage, and the virus retained its inability to form plaques at 35° C. There were no histological changes associated with the infection, and the basic growth parameters outlined in Fig. 1 were stable. The P17 thus recovered was demonstrably LCMV as it was neutralized by rabbit anti-LCMV serum. Therefore, we have isolated from the Armstrong strain of LCMV a stable variant that shows at least a 1000 fold reduction in virulence for adult mice when compared to the parental virus and that is capable of infecting mice and eliciting an apparently protective immune response.

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