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Susceptibility of Inbred Syrian Golden Hamsters (*Mesocricetus auratus*) to Lethal Disease by Lymphocytic Choriomeningitis Virus (42541)

EUGENE V. GENOVESI[1] AND C. J. PETERS

U.S. Army Medical Research Institute in Infectious Diseases, Department of Viral Pathogenesis and Immunology, Disease Assessment Division, Fort Detrick, Frederick, Maryland 21701

Abstract. An acutely lethal LCMV disease model has been established in the Syrian golden hamster (*Mesocricetus auratus*) in which lethality and disease are dependent upon both the inbred hamster strain and the LCMV strain. Young adult inbred, male and female, hamsters were tested for lethal-disease susceptibility by lymphocytic choriomeningitis virus (LCMV) strains, WE or Armstrong (ARM). With WE inocula, PD4 and MHA inbred hamsters were highly susceptible to a wasting disease. LVG and LHC inbred hamsters were intermediate in susceptibility; some of these animals died of wasting illness, and others exhibited minimal disease and survived. CB and LSH hamsters were highly resistant to any disease by WE. Mean survival times of susceptible hamsters given lethal WE inocula approximated 2.5 weeks and were not dependent on virus dose. By 1.5 weeks after WE inoculation wasting disease signs were notable and consisted of lethargy, progressive body weight loss, and diarrhea. The LCMV strain, ARM, was avirulent for all hamster strains, causing neither death nor disease. Hamsters surviving WE or ARM inoculation appeared healthy, produced LCMV antibody, and acquired resistance to further lethal WE challenge. Despite hamster-lethality differences, WE and ARM appeared comparably immunogenic for all hamster strains, based on host antibody titers. A number of other differences between the LCMV strains were, however, noted which could be relevant to virus virulence and lethality for hamster hosts. These included guinea pig lethality, temperature sensitivity, and plaque morphology.

Lymphocytic choriomeningitis virus (LCMV) infections of Syrian golden hamsters (*Mesocricetus auratus*) have long been recognized (1–23) and a concern in the epidemiology of human-LCMV outbreaks (10–22). Yet, previous studies on the mortality and virulence of LCMV for hamsters are not consistent. It is well documented that LCMV-inoculated adult and neonatal hamsters may undergo an inapparent, immunizing infection (1, 2, 5–7, 9–16, 20, 21). However, some reports allude to signs of illness among hamsters with immunizing nonlethal LCMV infections, but give few details (5, 10, 12). In addition to inapparent LCMV infections, virus-inoculated neonates may succumb to an acutely lethal illness (5, 9) or develop chronic vasculitis (9, 24). In LCMV-inoculated adult hamsters acute illness and death have also been observed (3, 4, 8, 13). But these later studies emphasized the benign nature of infection and the episodes of lethal illness among the LCMV-inoculated hamsters were not considered a consequence of viral etiology (1, 9, 13).

Confusion regarding LCMV lethality for hamsters may be due to the variability of virus strains and the genetic background of animals used in those studies. LCMV strains exhibit a marked range of biological and molecular heterogeneity which can affect their virulence potential for the appropriate host (16, 22, 23, 25–31). Furthermore, in this regard, the genetic background of hamster strains has proven to be a factor in the use of these animals as model hosts for studies of resistance and susceptibility to oncologic, microbial, and viral diseases (32–37). Because of such host–virus strain variability, a systematic study into the establishment of a uniformly lethal LCMV-disease model in the hamster was pursued. The susceptibility and resistance of young adult, female and male, inbred hamsters to LCMV mortality following intraperitoneal (ip) inoculation of either the WE strain, a virus highly virulent for rodents, or the Armstrong (ARM)

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strain, a virus relatively attenuated for rodents (1, 13, 16, 22, 23, 29, 31, 38–40), were determined. LCMV-inoculated animals were also monitored for overt disease signs, virus infection, and LCMV immunity.

Biologic differences and similarities between WE and ARM were also observed in this study.

Materials and Methods. *Virus stocks.* LCMV strains WE (39) and ARM (40) of recorded passage histories (23, 25) were obtained from Dr. W. E. Kirk (Dept. Microbiology, Medical Center, WV University, Morgantown, WV) as an infected mouse brain homogenate. Both LCMV strains were plaque-purified in a Vero-cell monolayer–agarose overlay system (see plaque assay for infectious LCMV) and propagated in confluent BHK-21 cell monolayers. Culture supernatants of virus were clarified and stored at −70°C. Parental stocks of LCMV were similarly prepared, but without any plaque purification.

Plaque assay for infectious LCMV. LCMV infectivity was measured in plaque-forming units (PFU) in a Vero-cell monolayer–agarose overlay system, readily used for LCMV and other arenaviruses (22, 23, 29, 35, 41, 42). Briefly, dilutions of virus or homogenized tissues of LCMV-inoculated animals were added to Vero-cell monolayers, followed by a medium-agarose overlay. After 7 days incubation in a humidified chamber with 5% CO_2 at 37°C, a medium-agarose overlay containing neutral red was added. Cultures were incubated for an additional 18 hr, and then plaques were counted. LCMV infectivity is expressed as $\log_{10}$ PFU.

Animals and preparation of tissue samples for virologic and immunologic assays. Young adult, female and male, Syrian golden hamsters reared at the Lakeview Hamster Colony (Newfield, NJ), were purchased at 8 to 10 weeks of age from Charles River Breeding Laboratories (Wilmington, MA). Inbred strains used were CB/Ss Lak, LHC/Lak, LSH/SsLak, MHA/SsLak, PD4/Lak, and the random colony bred Lak:LVG (SYR). The hamster strains are respectively referred to as CB, LHC, LSH, MHA, PD4, and LVG. Although the genetic homogeneity of these animals is not monitored, the pedigree of these hamster strains is assured by the commercial supplier.

Outbred Hartly strain guinea pigs (*Cavia porcellus*), 350 to 400 g weight were purchased from Buckberg Farms (Tomkins Cove, NY).

All animals were housed in P-3 laboratory containment facilities in air-filtered cages and fed and watered *ad libitum.* Sentinel uninoculated guinea pigs and hamsters were similarly maintained in the same facility in cages separate from LCMV-inoculated animals and were never found to have been infected or to have developed serum LCMV antibody. Before use, all animals were kept isolated for 2 weeks and had tested seronegative for LCMV.

Animals were inoculated ip with 0.2 ml of LCMV (either WE or ARM) in a serial decimal dose range of 0.3 to 6.3 $\log_{10}$ PFU. Unless indicated otherwise, plaque-purified LCMV stocks were used for inoculation, and animals were routinely monitored for body weight, signs of illness, and mortality. For infectious virus assay, blood samples were drawn from the retro-orbital sinus into heparinized tubes and stored at −70°C. Serum for LCMV antibody assays was collected from clotted blood kept at 4°C for 16 hr, then centrifuged at 1,000*g*, and stored at −20°C. Organs of sacrificed animals were aseptically weighed, homogenized, and clarified at 2,000*g* for 20 min. Prior to assay for infectious virus samples were stored at −70°C.

LCMV-antibody assays. Sera were heated at 56°C for 30 min and tested by an indirect immunofluorescent antibody test (IFAT) and an *in vitro* assay for neutralizing antibody used for arenaviruses (42, 43). For the IFAT, Vero cells infected with the parental ARM stock were dried and acetone-fixed on circular areas of microscope slides coated with Teflon (Cel-Line Associates, Minolta, NJ), and slides were stored at −20°C until use. Uninfected control Vero cells were similarly prepared on slides. For IFAT titration, serial twofold PBS dilutions of serum samples were reacted with uninfected control and LCMV-infected Vero cells on the slides for 20 min. Slides were then washed with PBS and reacted for another 20 min with an appropriate FITC–IgG conjugate of goat anti-hamster or anti-guinea pig γ-globulin (Cappel Laboratories, Cooper-Biomedical Inc., Malverne, PA). Slides were again PBS-washed and then mounted with a PBS–glycerol solution and a glass coverslip. Slides were examined for immunofluorescence using a Zeiss fluorescent light microscope, equipped with a 75-W zenon epi-illuminator lamp (Carl Zeiss Inc., West Germany). Endpoint serum LCMV-antibody IFAT titer was

determined as the highest dilution showing definite cytoplasmic granular fluorescence of LCMV-infected cells and was expressed as the mean reciprocal $\log_{10}$ dilution ± 1 SEM for sample groups. Serum samples that were negative for antibody by this procedure were recorded with IFAT titers of ≤0.7.

LCMV-neutralizing antibody was measured in a plaque reduction test by a constant-serum varied-virus protocol (42). The LCMV-neutralizing capacity of serum was determined using the Vero plaque assay of a standard LCMV preparation in the presence of a constant dilution of test serum. This titration was always conducted in parallel with a virus control in which the infectious titer of the standard LCMV was determined in the absence of any test serum. The standard LCMV preparation routinely used for this procedure was the parental WE stock, which had a 7.3 $\log_{10}$ PFU/ml titer. This virus was serially diluted fivefold in Eagle's minimal essential medium (EMEM) with 2% heat-inactivated fetal calf serum and 10% fresh normal guinea pig serum as a complement source. Each test serum was heat-inactivated and diluted 1:5 in EMEM. The diluted test serum was then added in equal volumes to each dilution of the WE virus standard and incubated at 37°C for 1 hr. Each serum–virus dilution was then applied to Vero-cell monolayers for plaque assay. Virus controls were run at the same time and simply consisted of the standard WE-virus dilutions mixed in equal volumes with EMEM devoid of any test serum. The resulting infectivity titers of the standard WE virus for each test serum and virus controls were recorded and used to calculate serum LCMV-neutralizing antibody titers. LCMV-neutralizing antibody titer was expressed as a mean $\log_{10}$ neutralization index (LNI) ±1 SEM for experimental animal groups. LNI was calculated as [($\log_{10}$ PFU of virus control) minus ($\log_{10}$ PFU of test serum)]. Serum samples that were negative for LCMV-neutralizing antibody were recorded with LNI titers of ≤0.7.

In vitro assessment of LCMV stocks for temperature sensitivity. Temperature sensitivity of LCMV stocks, plaque-purified and parental, was evaluated by two *in vitro* protocols. The first measured growth of infectious LCMV in Vero cells over a 72-hr period at 40 and 37°C; supernatants were titered at 37°C by the plaque assay above. In the second protocol, LCMV stocks were tested directly for plaque efficiency. All plaque assay conditions were the same as above except that test groups were incubated at 40°C for 7 days. Controls were incubated at 37°C for 7 days. In both test and control sets the medium-agarose overlay with neutral red was incubated for a subsequent 18-hr period at 37°C.

Results. Young adult female and male hamsters, aged 10 to 12 weeks, were observed for mortality and illness after ip inocula of decimal dilutions of either of the LCMV strains, WE or ARM (Fig. 1). Inbred female PD4 and MHA hamsters were highly susceptible to acutely lethal disease by WE. WE inocula of 2.3 $\log_{10}$ PFU and greater were lethal for all PD4 and MHA hamsters. Less than one-

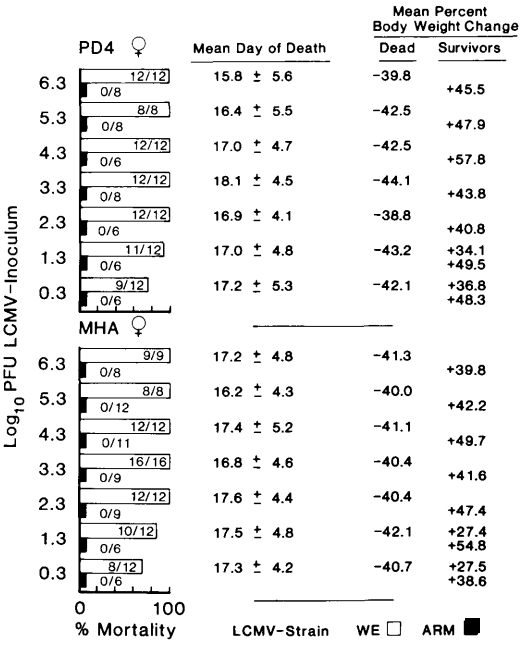

FIG. 1. Susceptibility of young adult female PD4 and MHA hamsters to lethal LCMV inoculation. Hamsters were inoculated ip with varied doses of LCMV strains, WE (empty bars) or ARM (filled bars), and observed for 42 days. Percentage mortality is shown by the bars and fractions are the ratio of dead animals to total number of animals inoculated with virus. Mean day of death (±1 SEM) does not include survivors. Mean percentage body weight change: Negative sign (−) denotes body weight loss of animals at the time of death. Positive sign (+) denotes body weight gain of survivors at 42 dpi. Both are shown as a percentage of mean animal body weights at the time of virus inoculation. Similar results were obtained with the males of these hamster strains.

third of PD4 and MHA hamsters survived 0.3 and 1.3 $\log_{10}$ PFU of WE. Regardless of WE dose, lethally inoculated hamsters expired at about 17 ± 5 days postinoculation (dpi) and had lost 40 to 45% of initial body weight by the time of death.

By 7 to 10 dpi all WE-inoculated PD4 and MHA hamsters exhibited signs of an acute wasting disease, initially characterized by ruffled fur and irritability and later with labored breathing, exhaustion, dehydration, weight loss, and evidence of diarrhea or "wet-tail" (44). There was no indication of paralysis or selective flaccid hind-limb weakness. Disease signs persisted in lethally inoculated animals to the time of death. The few PD4 and MHA that survived WE inocula of 0.3 and 1.3 $\log_{10}$ PFU had recovered from wasting illness and were healthy at 42 dpi with a respective 35 and 27% increase in body weight (Fig. 1). In contrast to WE inoculation, ARM did not kill PD4 and MHA hamsters. These animals appeared healthy and by 42 dpi they had gained 40 to 58% of initial body weight (Fig. 1).

CB and LSH inbred female hamsters were highly resistant to mortality by both WE and ARM (Fig. 2). These animals appeared healthy after inoculation and gained body weight. However, 1 of 12 CB female hamsters, inoculated with 6.3 $\log_{10}$ PFU of WE, died at 19 dpi. This animal did show wasting illness.

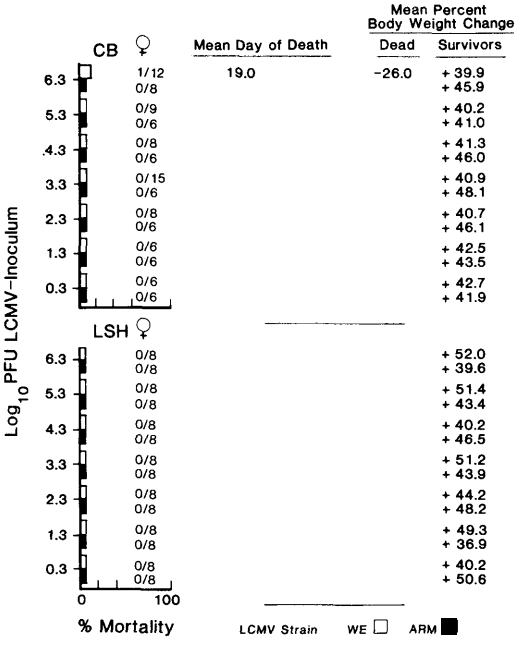

FIG. 2. Resistance of young adult female CB and LSH hamsters to lethal LCMV inoculation. Refer to Fig. 1 legend. Similar results were obtained with the males of these hamster strains.

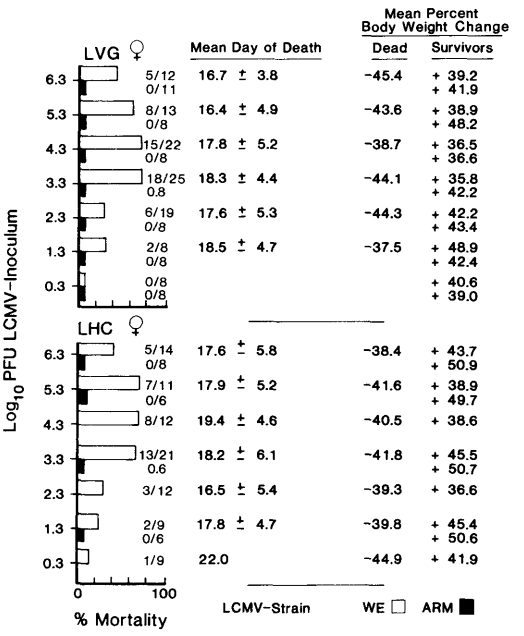

FIG. 3. Intermediate pattern of susceptibility and resistance of young adult female LVG and LHC hamsters to lethal LCMV inoculation. Refer to Fig. 1 legend. Similar results were obtained with the males of these hamster strains.

An intermediate mortality pattern was displayed by WE-inoculated LVG and LHC hamsters (Fig. 3). The mortality response of these animals to WE was not as uniform as the other strains (Figs. 1 and 2). WE inocula of 3.3, 4.3, and 5.3 $\log_{10}$ PFU resulted in 62 to 75% mortality, compared to 0.3, 1.3, 2.3, and 6.3 $\log_{10}$ PFU which resulted in 0 to 42% mortality. Lethally WE-inoculated LVG and LHC hamsters died about 17 ± 5 dpi regardless of virus dose and had wasting disease signs. At the time of death these animals lost 35 to 45% of initial body weight. WE-inoculated LVG and LHC survivors had minimal if any detectable signs of illness or body weight loss during the acute disease and mortality phase of their lethally inoculated counterparts. LVG and LHC hamsters were not killed by ARM. These ARM-inoculated animals appeared healthy with significant weight gain by 42 dpi (Fig. 3).

Young adult male hamsters exhibited disease and mortality response patterns to graded WE inocula which were no different from those of females of the same respective strains. Also, all male hamsters were refractory to illness and lethality by ARM, as were the females (data not shown).

The time course of mortality and body weight change of young adult female hamsters was followed after ip WE challenge of 3.3 $\log_{10}$ PFU (Fig. 4). All MHA hamsters died 9 to 24 dpi, with a mean death time of 16.6 ± 5.0 days. Progressive body weight loss by these animals was obvious by 14 dpi. Body weights of individual MHA hamsters at death are shown with a mean of 60.6 ± 5.8 g, a 37.8% decrease from the time of WE inoculation.

Mortality and body weight changes of LVG hamsters, intermediate in susceptibility and resistance to lethal disease by WE, are also presented (Fig. 4). Of 13 inoculated LVG hamsters, 8 died between 11 and 26 dpi with a mean death time of 17.8 ± 3.6 days. Mean body weight declined between 1 and 3 weeks after virus challenge. The 8 lethally inoculated LVG hamsters were markedly ill with a 42.3% body weight decrease at the time of death. The 5 nonlethally inoculated animals exhibited minimal to no illness with little body weight loss. By 28 dpi these 5 animals had recovered and by 35 dpi they had gained 20.8% of initial body weight.

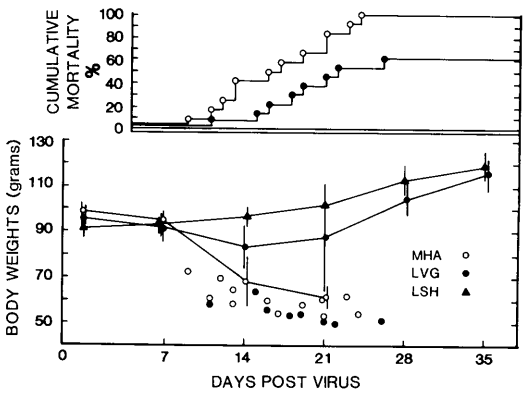

FIG. 4. Time course of mortality and body weight changes of young adult female hamsters, following ip inoculation of 3.3 $\log_{10}$ PFU of WE. Observations are based on groups of 12 MHA (empty circles), 13 LVG (filled circles), and 12 LSH (filled triangles) animals. Top: Daily cumulative mortality for MHA and LVG hamsters. No LSH hamsters died. Bottom: Body weights (±1 SEM) of the respective hamsters at weekly intervals are connected by the lines. Shown as unconnected points are body weights of individual hamsters of each strain at time of death.

None of the WE-inoculated LSH hamsters had signs of illness. By 35 dpi they had gained 32.4% of original body weight (Fig. 4).

Lethally and nonlethally LCMV-inoculated hamsters had been routinely monitored for their virologic and immunologic status. Regardless of inbred strain, by 7 dpi, all WE- and ARM-inoculated animals were viremic and underwent LCMV seroconversion as determined by IFAT (data not shown). Hamsters that survived WE or ARM were not viremic by 42 dpi (Tables I–III) and had no detectable infectious virus or LCMV antigens in their organs (data not shown).

LCMV immunity of young adult female hamsters, surviving primary ip WE and ARM inoculation, is shown for strains which are highly resistant (Table I), intermediate in resistance (Table II), and highly susceptible (Table III) to mortality and disease by WE. At 42 dpi surviving hamsters had IFAT and LNI LCMV-antibody titers which varied with primary ip inoculum size. Animals given WE or ARM inocula of 2.3 to 3.3 $\log_{10}$ PFU and greater had comparable titers, with IFAT $\geq$ 3.5 and neutralizing-antibody titers of LNI $\geq$ 2.5. At lower WE and ARM inocula, 0.3 to 2.3 $\log_{10}$ PFU, IFAT and LNI titers were $\geq$1.0 to $\leq$3.0, and $\geq$1.0 to $\leq$2.5, respectively. Furthermore, regardless of strain, all hamster survivors of primary WE or ARM inocula at 42 dpi were resistant to mortality by ip WE challenge of 4.3 $\log_{10}$ PFU. All these animals remained healthy, LCMV-antibody positive, and nonviremic at 2 months after this WE challenge (data not shown).

Note also that at the time LCMV survivor hamsters were WE-challenged, uninoculated age-matched control hamsters of each strain were also inoculated ip with 4.3 $\log_{10}$ PFU of WE (Tables I–III). These animals, following a 7-week age increment, maintained their respective host strain-dependent mortality patterns of susceptibility to lethal WE disease.

Parental and plaque-purified LCMV preparations of WE and ARM stocks were tested for differences in traits (Table IV) that may be relevant to their hamster-lethal potential. Lethality for guinea pigs was examined. Serial decimal dilutions of the respective LCMV strains were inoculated ip into groups of six

TABLE I. SURVIVIAL AND LCMV IMMUNITY OF LCMV DISEASE-RESISTANT HAMSTERS AFTER PRIMARY LCMV INOCULATION

Primary-ip LCMV inoculum		LSH hamster strain[a]			
			LCMV immunity		
			LCMV antibody[c]		
LCMV strain	$\log_{10}$ PFU	Survival ratio[b]	IFAT	LNI	Rechallenge resistance[d]
WE	6.3	8/8	3.8 ± 0.3	2.6 ± 0.3	8/8
	5.3	8/8	3.6 ± 0.2	2.7 ± 0.3	8/8
	4.3	8/8	3.9 ± 0.3	2.6 ± 0.3	n .t.[e]
	3.3	8/8	3.7 ± 0.3	2.8 ± 0.3	8/8
	2.3	8/8	2.9 ± 0.6	2.8 ± 0.4	n.t.
	1.3	8/8	2.6 ± 0.3	1.8 ± 0.3	8/8
	0.3	8/8	2.0 ± 0.5	1.3 ± 0.4	8/8
ARM	6.3	8/8	3.5 ± 0.5	2.9 ± 0.3	8/8
	5.3	8/8	3.8 ± 0.4	3.2 ± 0.3	n.t.
	4.3	8/8	3.8 ± 0.4	3.1 ± 0.4	n.t.
	3.3	8/8	3.7 ± 0.4	2.6 ± 0.3	8/8
	2.3	8/8	3.6 ± 0.3	2.8 ± 0.2	n.t.
	1.3	8/8	3.0 ± 0.5	2.2 ± 0.3	n.t.
	0.3	8/8	2.4 ± 0.5	1.4 ± 0.3	8/8
Uninoculated age-matched controls			≤0.7	≤0.7	6/6

[a] Young adult female LSH hamsters are resistant to lethal disease by WE. Similar results were observed for female CB hamsters.

[b] Number of animals alive at 42 dpi to total number given primary ip inocula of WE or ARM.

[c] At 42 dpi prior to WE challenge, serum and blood samples were drawn from all animals and individually tested for LCMV antibody by IFAT and LNI, and for infectious virus. All serum and blood samples were negative for infectious LCMV.

[d] At 42 dpi, LCMV survivors, or randomly selected subgroups of survivor animals with representative antibody titers, and uninoculated age-matched control hamsters were WE-challenged ip with 4.3 $\log_{10}$ PFU and observed 60 days for death. Fractions show survival ratios for these animals.

[e] Not tested.

or more animals per virus dose in the range of 0.3 to 7.3 $\log_{10}$ PFU. Plaque-purified and cloned WE stocks killed all guinea pigs inoculated with 0.3 $\log_{10}$ PFU or more virus within 7 to 10 days. The ARM stocks were not lethal even at 7.3 $\log_{10}$ PFU. All ARM-inoculated guinea pigs survived beyond 42 days, were healthy and nonviremic, and had seroconverted to LCMV as determined by IFAT and LNI (data not shown).

Both LCMV strains were also tested for temperature sensitivity (Table IV). Plaque-purified and cloned WE stocks were relatively unaffected in growth and plaque efficiency *in vitro* at 40°C. In three separate determinations at 40°C WE growth in Vero cells over a 72-hr period and virus plaque formation in Vero cells were never below 80% of control virus titers at 37°C. However, growth and plaque efficiency of parental and cloned ARM stocks were totally suppressed at 40°C.

Morphologically, the plaques of both virus strains in Vero cells under a medium-agarose overlay at 37°C were also different (Table IV). WE plaques were relatively large, 3–5 mm diameter, and clear. ARM plaques appeared small and pinpoint with diameters of 0.5–3 mm.

Discussion. In susceptibile hosts of different mammalian species LCMV infections can produce a variety of clinical patterns which include acute illness with either a lethal or nonlethal outcome, late and chronic disease, or no overt disease (2, 16, 18, 38–40, 45–47). Most studies with hamsters have emphasized an inapparent LCMV-infection pattern (1, 2, 5–7, 9–16), although there were reports of acute and chronic illness (3, 4, 8, 9, 13). We

TABLE II. SURVIVAL AND LCMV IMMUNITY OF PARTIALLY LCMV DISEASE-SUSCEPTIBLE HAMSTERS AFTER PRIMARY LCMV INOCULATION

Primary-ip LCMV inoculum		LVG hamster strain[a]			
			LCMV immunity		
			LCMV antibody[c]		
LCMV strain	$\log_{10}$ PFU	Survival ratio[b]	IFAT	LNI	Rechallenge resistance[d]
WE	6.3	7/12	3.8 ± 0.2	2.8 ± 0.4	5/5
	5.3	5/13	3.8 ± 0.3	3.2 ± 0.4	5/5
	4.3	7/22	3.7 ± 0.4	n.t.[e]	4/4
	3.3	7/25	3.7 ± 0.3	2.8 ± 0.3	7/7
	2.3	3/19	2.8 ± 0.5	1.5 ± 0.3	13/13
	1.3	6/8	2.7 ± 0.3	1.4 ± 0.4	n.t.[e]
	0.3	8/8	2.1 ± 0.3	0.8 ± 0.2	n.t.
ARM	6.3	1/11	3.9 ± 0.4	2.6 ± 0.4	n.t.
	5.3	8/8	3.9 ± 0.3	3.0 ± 0.3	8/8
	4.3	8/8	3.9 ± 0.3	3.1 ± 0.3	8/8
	3.3	8/8	3.8 ± 0.3	2.8 ± 0.2	8/8
	2.3	8/8	3.3 ± 0.4	2.5 ± 0.3	n.t.
	1.3	8/8	2.6 ± 0.5	1.8 ± 0.4	8/8
	0.3	8/8	1.8 ± 0.3	1.3 ± 0.3	8/8
Uninoculated age-matched controls			≤0.7	≤0.7	4/11

[a] Young adult female LVG hamsters exhibit an intermediate pattern of susceptibility and resistance to lethal disease by WE. Similar results were observed for female LHC hamsters.
[b–e] Refer to Table I.

have shown that the susceptibility of young adult Syrian golden hamsters to illness and death is dependent upon both the host strain and the virus strain (Figs. 1–3). This observation may resolve some confusion in the literature regarding the inherent susceptibility of these rodents to lethal LCMV disease (1–16, 20, 21). While the ARM strain was not virulent for any hamster strain tested, WE inocula resulted in three susceptibility patterns (Figs. 1–3). Inbred PD4 and MHA hamsters were highly susceptible to acute illness and mortality by WE. LHC and LVG hamsters were intermediate or partial in susceptibility. CB and LSH inbred hamsters resisted death and disease by WE.

The dominant clinical manifestation of LCMV disease was weight loss which occurred in all lethally WE-inoculated hamsters (Figs. 1–4). Weight loss was evident between 7 and 14 days after WE inoculation and was progressive. Inoculation of WE into resistant CB or LSH hamsters and inoculation of the ARM strain into all hamsters caused no weight loss or disease signs (Figs. 1–4). Hamsters of strains that were intermediate in susceptibility to WE either developed lethal wasting disease typical of WE-susceptible PD4 and MHA hosts or survived with little or no overt illness.

Another major sign of fatal hamster LCMV infection was diarrhea associated with "wet-tail" (44) and dehydration. This disease sign was confined to WE-inoculated susceptible hamsters (PD4 and MHA) and to the lethally inoculated subset of hamsters of intermediate susceptibility to WE (LVG and LHC). "Wet-tail" had been described for occasional hamsters that died after LCMV inoculation (1, 9), but its significance had remained obscure. This may have been due in part to a low incidence of LCMV deaths in those studies, and also to the unconfirmed suspicion that the cause of death was the bacterial agent of transmissible ileal hyperplasia (TIH) (48). Our ongoing gross and histologic studies will assess the possible involvement of TIH in acutely lethal LCMV hamster disease.

As LCMV is highly infectious for hamsters (1–16, 19–21), one might possibly expect that with increasing doses of virus, mortality would

TABLE III. SURVIVAL AND LCMV IMMUNITY OF LCMV DISEASE-SUSCEPTIBLE HAMSTERS AFTER PRIMARY LCMV INOCULATION

Primary-ip LCMV inoculum		MHA hamster strain[a]			
			LCMV immunity		
			LCMV antibody[c]		
LCMV strain	$\log_{10}$ PFU	Survival ratio[b]	IFAT	LNI	Rechallenge resistance[d]
WE	6.3	0/9	—	—	—
	5.3	0/8	—	—	—
	4.3	0/12	—	—	—
	3.3	0/16	—	—	—
	2.3	0/12	—	—	—
	1.3	2/12	2.9 ± 0.5	1.9 ± 0.3	2/2
	0.3	4/12	2.1 ± 0.4	1.3 ± 0.2	4/4
ARM	6.3	8/8	3.9 ± 0.3	3.2 ± 0.3	8/8
	5.3	12/12	3.8 ± 0.3	2.8 ± 0.3	8/8
	4.3	11/11	3.8 ± 0.3	2.7 ± 0.3	6/6
	3.3	12/12	3.8 ± 0.2	2.8 ± 0.3	8/8
	2.3	9/9	3.1 ± 0.2	2.5 ± 0.3	n.t.[e]
	1.3	6/6	3.0 ± 0.4	n.t.[e]	6/6
	0.3	6/6	2.2 ± 0.9	n.t.	6/6
Uninoculated age-matched controls			≤0.7	≤0.7	0/7

[a] Young adult female MHA hamsters are susceptible to lethal disease by WE. Similar results were observed for female PD4 hamsters.
[b–e] Refer to Table I.

increase and survival time would decrease in susceptible hosts. However, these virus–dose relationships were not clearly observed. All hamsters of highly WE-susceptible strains, PD4 and MHA, died after inoculation of 2.3 $\log_{10}$ PFU or larger doses of WE. A few, however, did survive doses of 0.3 or 1.3 $\log_{10}$ PFU (Fig. 1). In contrast WE failed to kill more than 75% of LVG and LHC hamsters of intermediate WE susceptibility at any dose level

TABLE IV. DIFFERENCES AND SIMILARITIES BETWEEN THE HAMSTER LETHAL AND NONLETHAL LCMV STRAINS[a]

WE	ARM
1. Hamster lethal: Lethal disease is host strain dependent.	Hamster nonlethal: All hamster strains are resistant to mortality and disease.
2. Immunogenicity: Elicits antibody response comparable to ARM in lethal-resistant hamster strains and in the few survivors of lethal-susceptible hamster strains.	Immunogenicity: Similar to WE in virus-specific antibody induction; cross-protects susceptible inbreds against lethal LCMV.
3. Guinea pig lethal by ip route: $LD_{50} < 10^{0.3}$ PFU.	Guinea pig nonlethal by ip route: $LD_{50} > 10^{7.0}$ PFU.
4. Not temperature sensitive: Plaque efficiency and virus growth at 40°C is at least 80% of that at 37°C.	Temperature sensitive: Plaque efficiency and virus growth significantly inhibited at 40°C.
5. Plaque size and morphology: 3 to 5 mm diameter, large, clear.	Plaque size and morphology: 0.5 to 3 mm diameter, small, pinpoint.

[a] Parental and plaque-purified preparations of each respective LCMV strain (WE or ARM) were not different in the indicated attributes.

(Fig. 3). Indeed for these animals mortality was lower at low virus doses (0.3 and 1.3 $\log_{10}$ PFU) and the highest dose (6.3 $\log_{10}$ PFU). For all hamsters with lethal WE infection mean survival times were usually 16 to 19 days and this did not change with virus inoculum size (Figs. 1, 3, 4). Adult mice given lethal intracranial LCMV inocula also showed a lack of dependence of survival time on virus dose and a similar "prezone" effect in which higher LCMV doses resulted in lower mortality (16, 45, 49, 50).

The virologic and immunologic status of LCMV survivor hamsters (Tables I–III) suggested that both WE and ARM produced acute infections and are highly immunogenic. LCMV-antibody titers (IFAT and LNI) at 42 dpi tended to be higher with increasing virus inoculum size, but did not appear to depend on virus strain or hamster strain. Similar relationships between virus dose and host antibody (51) and T-lymphocyte (52) responses to LCMV have been reported in inbred mice. In addition to the LCMV-antibody response to WE or ARM, solid host resistance to lethal LCMV challenge was induced in WE-susceptible hamsters by ARM inoculation. This demonstrable cross-protective immunizing capacity of the nonlethal ARM for hamsters was also noted in guinea pigs (16). It may be concluded that hamster susceptibility to lethal WE disease is not due to the inability of these animals to mount a humoral antibody response to LCMV or to acquire resistance to the virulent virus strain by exposure to an attenuated LCMV inoculum. These observations focus on the LCMV-immune response as a critical area requiring further study in the hamster.

Virus–host interactions involved in the clinical outcome of hamster LCMV infections are not well understood. Immunopathogenetic mechanisms, that underlie murine LCMV-infection models of acutely lethal visceral or neurologic disease, or the chronic immune-complex disease of persistently viremic carrier hosts (16, 17, 22, 45, 47, 49, 50, 53–55) has been invoked to explain the course of hamster LCMV-infections (5, 9, 15–17, 20–22, 54). In the mouse, acutely lethal LCMV disease results from a virus-specific host immune-mediated process in which an effective T-lymphocyte response eliminates viral infection at the expense of the host. In the absence of this response, mice do survive, but remain persistently infected as life-long LCMV carriers. However, murine LCMV carriers develop an ineffective humoral antibody response that leads to chronic immunecomplex disease.

Our hamster LCMV data and results of several other studies (1, 5, 9–14) suggest a different host–virus relationship. Surviving LCMV-inoculated adult hamsters had no apparent illness, were aviremic, had circulating LCMV-neutralizing antibody, and were immune to virus challenge (Tables I–III). From further work in our laboratory, tissues of LCMV hamster survivors were shown to be free of infectivity and viral antigens as well (unpublished observations). These observations are more consistent with the pathogenesis that occurs in adult mice following the extraneural inoculation of attenuated LCMV. In these animals acute infection is resolved by an active host antiviral immune response without any illness (16, 56, 57). However, it must still be determined whether lethal LCMV disease of susceptible inbred hamsters is immune-mediated or a virus-induced pathophysiologic process (58, 59).

The plaque-purified ARM and WE stocks used in this study were not different from their respective parental virus stocks in the properties listed in Table IV. As plaque-purified ARM and WE were markedly disparate in hamster lethality, so were the respective parental stocks. Moreover, these virus preparations maintained their differing virulence characteristics for guinea pigs (16, 23, 29). Plaque size and morphology differences between ARM and WE (Table IV) also conformed to published accounts (23, 25).

Of further interest was the *in vitro* growth sensitivity of these LCMV strains to temperature. Nonlethal ARM stocks were very sensitive to temperature inhibition at 40°C, whereas WE stocks were not (Table IV). Temperature sensitivity may be a significant correlate to LCMV virulence in hamsters, as well as other species, and ought to be further evaluated with respect to LCMV pathogenesis (11, 22, 27, 28). Primary LCMV infection induces a febrile response in guinea pigs, monkeys, and man (11, 16, 18, 39, 40, 46). Such a febrile host response could mediate protection (60) against potential lethal disease by avirulent, immunizing LCMV strains such as ARM. A

fever mechanism may also explain the unusual report of a nonlethal, immunizing infection of adult mice following intracranial inoculation with a cold-adapted ARM variant (28).

The relevance of the properties listed in Table IV, as well as other LCMV characteristics (16, 18, 22, 29, 30) not examined here, to the virulence and immunogenicity of the many different LCMV strains for their hosts, including hamsters, is at best speculative. In this regard current developments in LCMV-genome analysis (22, 23, 31, 41) ought to be helpful in the identification of virus genes that control virulence and immunogenic determinants.

Until now there was no well-defined hamster model of lethal disease by LCMV (1–17, 20, 21). By ip challenge with the LCMV stain, WE, the relative susceptibility of hamster stains to lethal wasting disease was established. Although the genetic mode of inheritance of host susceptibility and resistance to this virus was not examined by appropriate breeding experiments, the survey of adult inbred female (Figs. 1–3) and male (data not shown) hamsters to LCMV mortality suggests that susceptibility is autosomal and not coexpressed with known alleles of the major hamster histocompatibility complex, Hm-1 (61). A goal in our ongoing inquiry into hamster LCMV infections is to find host genes and functions that correlate with hamster strain susceptibility or resistance to LCMV disease.

This study also extends the utility of inbred hamsters as an animal model to investigate genetic, physiologic, and immunologic parameters of host susceptibility to viral disease (32, 33) as shown for VSV (34) and Pichinde (35), another arenavirus. It may also provide a realistic experimental small animal system for research into human arenavirus pathogenesis and immunology, and for the development of prophylactic and therapeutic strategies against human arenavirus diseases (14, 17, 18, 22).

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