Variation Between Strains of Hamsters in the Lethality of Pichinde Virus Infections

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Infection by Pichinde virus, a member of the arenavirus group, was studied in Golden Syrian hamsters (*Mesocricetus auratus*) with regard to possible mechanisms of resistance to virus infection in adult hamsters. Two hamster strains were found to differ in their susceptibility to lethal Pichinde virus infection. LVG/Lak randomly bred hamsters were found to be 100% susceptible to low doses of Pichinde virus during the first 6 days of life, but after 8 days of life, mortality was uncommon. Peak virus titers in the serum of animals infected at 3 days of life were 4 logs greater than in animals infected at 12 days. MHA/Lak inbred hamsters, in contrast, were found to be susceptible to lethal virus infection both as newborns and as adults. Peak virus titers of greater than 10^8 plaque-forming units/ml were observed in serum 8 days after infection of adult MHA hamsters as compared with less than 10^3 plaque-forming units/ml in the serum of adult LVG hamsters. Cultured primary kidney cells and peritoneal macrophages from either hamster strain supported Pichinde virus replication equally well in vitro. Antibodies to the complement-fixing antigens and to antigens at the surface of virus-infected cells were produced by both strains of hamsters. Cyclophosphamide immunosuppression rendered adult LVG animals susceptible to lethal infections, and virus grew to high titers in the treated animals. These findings suggest that immunological factors that appear early in life in LVG hamsters and are deficient in MHA hamsters limit Pichinde virus infection. Unlike previously reported arenavirus diseases, the observations suggest that death is produced by a direct viral effect and not through immunopathological mechanisms.

A variety of host responses to arenavirus infections have been noted, and immune-mediated pathological changes appear to contribute significantly to these responses (21). Major factors that influence the outcome of the virus-host interaction are age at infection, route of inoculation, biological properties of the virus strain, and the genetic composition of the host. Genetic influences on resistance and susceptibility to viral infections have long been appreciated (23). Among the arenaviruses, however, only lymphocytic choriomeningitis (LCM) virus infection in mice has been studied with respect to genetic control of resistance (22). In these studies, relative susceptibility (a dominant trait) and relative resistance were linked to the major histocompatibility (H-2) locus. It was suggested that a genetic influence on the immune response to LCM virus affected the outcome of the infection. Mice with vigorous immune responses were more likely to die than

other mice since death in acute LCM virus infection is a consequence of immune-mediated pathological changes (18).

While examining the age-related lethality of hamsters to infections by Pichinde virus, we found differences between two strains of adult hamsters. This report documents the variations in the outcome of Pichinde virus in adult hamsters of two strains and provides evidence that suggests that lethality results from an inability to control virus replication.

MATERIALS AND METHODS

Virus and virus assays. Pichinde virus, strain AN3739, was originally obtained from Carlos Sanmartin (25). The virus, isolated from the blood of an *Oryzomys albicularis* rodent, was passaged 12 times in newborn hamster brain, 3 times in Vero cells, and then 6 times in BHK-21 cells. Stock virus was prepared in monolayer cultures of BHK-21 cells and assayed as previously described (19). Infectious virus in the blood, serum, and solid organs of infected animals was quantitated by the plaque assay method (19). Solid organs were weighed and then ground in a mortar with sufficient Eagle minimal

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essential medium (MEM) to make a 10% (wt/vol) suspension. The suspensions were clarified by centrifugation at 1,500 rpm for 15 min, and the supernatant fluids were titered. Titrations were performed on two animals at each time point. Virus titers were expressed as plaque-forming units (PFU) per gram of solid organs, or per milliliter of serum or blood. All virus-containing samples were held at -85°C prior to titration.

Primary cell cultures. Primary kidney cells were cultured from minced, trypsinized kidneys of 3-day-old and adult hamsters. The cells were seeded into 25-cm² plastic tissue culture flasks and incubated in MEM supplemented with 10% heat-inactivated fetal calf serum. Flasks were sealed and cultured at 37°C . The medium was changed after the first 24 h of incubation, and cells reached confluency 3 days after seeding.

Peritoneal exudate macrophages were obtained from hamsters stimulated 72 h earlier by intraperitoneal injection of 5 ml of sterile mineral oil. Cells were harvested aseptically by washing the peritoneal cavity repeatedly with Hanks balanced salt solution. The harvested cells were washed twice with Hanks balanced salt solution and counted in a hemocytometer. The cells were infected in suspension with 1 to 3 PFU of Pichinde virus per viable nucleated cell. Adsorption was for 60 min at 4°C , after which the cells were washed with and resuspended in MEM supplemented with 10% heat-inactivated fetal calf serum. The resuspended cells were seeded into glass tissue culture tubes at a density of 2×10^6 cells per ml in a volume of 1 ml. Incubation was at 37°C in an atmosphere of 5% CO_2 . Between 85 and 95% of the nucleated cells in cultures established in this way were morphologically similar to macrophages.

Antibody assays. Complement-fixation tests for detection of antibody to Pichinde virus were carried out in microtiter as described elsewhere (5). Antigens were prepared from BHK-21 cells infected with Pichinde virus (5). Titers of antibody were expressed as the reciprocal of the highest serum dilution giving >50% inhibition hemolysis.

Antibodies to virus antigens appearing on the surface of infected cells were detected by blocking the binding of ^{125}I -labeled anti-Pichinde virus immunoglobulin G (IgG). IgG was purified from hamster immune serum by three precipitations with $(\text{NH}_4)_2\text{SO}_4$ at 50, 40, and 35% saturation followed by ion-exchange chromatography on a diethylaminoethyl-cellulose column equilibrated with 0.05 M sodium phosphate buffer, pH 7.4. The first protein peak eluted was shown by immunoelectrophoresis to contain only IgG. This peak was concentrated to 4 mg of protein per ml. Portions of 40 μg were iodinated by using chloramine T (10). The iodinated IgG was passed through a G-25 Sephadex column to eliminate unbound ^{125}I . The test was performed by aliquoting 1×10^6 monodispersed cells into siliconized tubes (12 by 75 mm). The cells were pelleted at 1,000 rpm for 3 min, the supernatant was drained from the tubes, and 50 μl of blocking antiserum diluted in phosphate-buffered saline was added to each tube. After 30 min at 37°C , the cells were

pelleted, the supernatant fluid was removed, and 50 μl of ^{125}I -labeled IgG was added. The cells were again held at 37°C for 30 min, after which they were washed five times with phosphate-buffered saline containing 2% heat-inactivated fetal calf serum. Before the final centrifugation, the resuspended cells were transferred to new tubes and, after the cells were pelleted, the supernatants were removed and the radioactivity remaining with the cells was determined with a Beckman automatic gamma counter. Results of blocking were expressed as the percentage of maximum ^{125}I -labeled IgG binding; the counts per minute in the presence of test serum were divided by the counts per minute in presence of normal serum times 100.

Animals. Hamsters used in this study were obtained from Charles River/Lakeview. The strains used were LVG/Lak randomly bred and MHA/Lak inbred hamsters. Adult animals were obtained at 5 to 9 weeks of age and used at 6 to 12 weeks of age. Pregnant females were obtained at 12 to 13 days of gestation, and litters were usually born on day 17. Newborns were used in the first 72 h of life unless otherwise indicated. Suckling hamsters were inoculated subcutaneously on the back with 0.05 ml of virus dilution. Observations for illness and death were made daily, starting on day 2 after inoculation. All animals were housed in wire-topped polycarbonate cages and fed Purina Chow and water ad lib.

Cyclophosphamide treatment. Cyclophosphamide (Procytox, Horner Ltd., Montreal) was prepared at a concentration of 10 mg/ml in saline immediately prior to use. Hamsters were treated 3 days after infection by intraperitoneal injection of 100 or 150 mg of cyclophosphamide per kg, as indicated in the text. Noninfected hamsters that were given the drug were included in each experiment for control purposes, and no lethality due to cyclophosphamide treatment alone was observed.

RESULTS

Lethality of Pichinde virus in LVG and MHA hamsters. Pichinde virus produced a lethal infection in neonatal hamsters of both strains. Figure 1 shows the mortality of infected LVG hamsters. The virus killed 100% of the animals infected on the day of birth, and 50% of the animals were dead by day 10 after infection. Infection at 4 days of age again killed all the animals, with approximately the same duration between infection and death. The response of animals at 6 days differed in that the duration between infection and death was prolonged; 50% of the animals were dead by day 25. Lethality was unusual in LVG hamsters infected at 8 days of age or older.

Although newborn MHA hamsters died after Pichinde virus infection in a manner similar to newborn LVG hamsters (data not shown), the ability to survive the infection did not develop with age in this strain of hamsters. This is illustrated in Fig. 2, and it can be seen that 7-

FIG. 1. Mortality among LVG hamsters after Pichinde virus infection at various ages. Hamsters were infected by subcutaneous incubation of 500 PFU of Pichinde virus on days 0 (○), 4 (□), 6 (△), and 8 (◆) after birth and then observed daily for death.

FIG. 2. Mortality among 7-week-old MHA hamsters after intraperitoneal infection with various doses of Pichinde virus. Virus doses were: ○, 3.5×10^6 PFU; △, 3.5×10^4 PFU; and □, 35 PFU/animal.

week-old MHA hamsters were killed with as little as 35 PFU of virus. Except for animals receiving 35 PFU of virus, the duration between infection and death in the 7-week-old MHA hamsters was similar to that of LVG hamsters infected at 0 and 4 days of age with similar doses of virus. Thus, unlike LVG hamsters, the MHA hamsters did not acquire a resistance to the lethality of the virus infection.

Replication of virus in infected hamsters. The replication of Pichinde virus was assessed in LVG hamsters infected at different ages by assaying for infectious virus in organs and blood. Figure 3 shows that maximum virus titers were reached 6 to 8 days after infection, and higher titers were observed in LVG hamsters infected at 3 days of age than at 6 days of age. In another experiment, LVG hamsters of various ages were infected, and viremia was

quantitated at 4 and 9 days after infection (Table 1). At 4 days after infection, 2-log_{10} -higher titers of virus were present in animals infected at 3 days of age than in animals infected at older ages; however, the virus titers at 4 days were not substantially different among animals infected at 6, 12, or over 30 days of age. By 9 days after infection, virus titers correlated with the age at infection; highest titers were observed among animals infected at 3 days of age, whereas the lowest titers occurred among animals infected as adults. These observations suggest that maturing LVG animals acquire the ability to limit virus replication, and limited virus replication correlates with survival.

A possible explanation for the lethal outcome of Pichinde virus infections in MHA hamsters is that these animals do not acquire the ability to limit virus replication. To examine this possibility, adult MHA and LVG hamsters were infected intraperitoneally with 2×10^5 PFU of Pichinde virus and various organs were assayed for virus at intervals after infection. Table 2 shows that adult MHA hamsters do not limit virus replication in the same manner as

FIG. 3. Titers of Pichinde virus recovered from (A) spleen, (B) liver, (C) kidney, and (D) blood of LVG hamsters infected with 400 PFU subcutaneously at 3 (●) or 6 (○) days of age.

TABLE 1. Serum titers of Pichinde virus after infection of LVG hamsters at various ages

Age of inoculation (days)	Log ₁₀ virus titer (PFU/ml) at:	
	Day 4 ^a	Day 9
3	5.8	8.4
6	3.8	5.3
12	3.2	4.1
Adult (>30)	3.0	<3.0

^a Day after inoculation.

LVG hamsters. The serum virus titers were similar for both strains of hamsters on day 4 after infection; however, by 8 days after infection $5 \log_{10}$ more virus was present in the serum of MHA hamsters than in LVG hamsters. In every organ tested the peak virus titer reached in MHA hamster was greater than that of their LVG counterparts.

Virus replication in cultured cells. The acquired ability to limit virus replication by LVG hamsters could represent either an age-dependent inability of the animal's cells to support virus replication or an age-related acquisition of immune responsiveness to the virus. To distinguish between these possibilities, the replication of Pichinde virus in primary cell cultures of the two strains was examined. The replication of the virus in primary kidney cells cultured from 3-day-old and adult hamsters is shown in Fig. 4. No differences in growth of the virus were noted between strains. Virus production was greater in cultures of cells obtained from 3-day-old animals than from cells obtained from adult animals; however, the cultures from 3-day-old animals contained more cells than did cultures from adults.

Peritoneal exudate macrophages from adult LVG and MHA hamsters were also tested for their ability to support Pichinde virus replication. Peritoneal exudate cells from oil-stimulated animals were harvested, infected with virus, and cultured as described above. Figure 5 shows that the virus grew equally well in the peritoneal exudate cells of both strains of hamsters. Thus, no inherent differences were found between LVG and MHA hamsters in their abil-

FIG. 4. Growth of Pichinde virus in primary kidney cell cultures established from 3-day-old and adult MHA and LVG hamsters. Cultures established from 3-day-old and adult MHA and LVG hamsters were infected with approximately 1 to 3 PFU of Pichinde virus per cell. After adsorption, cells were overlaid with maintenance medium and incubated at 37°C. Medium was changed at 24-h intervals, and samples taken at the time indicated represent virus produced in the previous 24 h. Symbols: ▲, 3-day MHA; ●, 3-day LVG; △, adult MHA; ○, adult LVG.

ity to support Pichinde virus replication as assessed in cultures of kidney cells and peritoneal exudate cells.

Effect of immunosuppression by cyclophosphamide. Immunosuppression by cyclophosphamide was used to evaluate the possible role of immune responsiveness in the virus-induced mortality. A single dose of cyclophosphamide was administered 3 days after infection of both hamster strains with Pichinde virus. The results of a typical experiment are shown in Fig. 6. Cyclophosphamide immunosuppression effectively eliminated the age-acquired resistance of LVG hamsters; animals infected with Pichinde virus and treated with cyclophosphamide died, with approximately 50% of the deaths occurring by day 17 after infection. The mortality of MHA hamsters was not appreciably changed by the immunosuppressant. Virus assays of sera collected 10 days after injection of Pichinde virus failed to reveal virus at a 1:100 dilution of sera from LVG hamsters given virus alone, whereas titers of 9.2×10^7 to 1.5×10^8

TABLE 2. Pichinde virus titers in various organs of LVG and MHA hamsters after infection

Organ	Hamster strain	Virus titer ^a at day after inoculation:				
		1	4	8	11	15
Blood (serum)	MHA	<2 ^b	3.1	8.1	7.2	— ^c
	LVG	<2	3.1	2.9	1.5	—
Kidney	MHA	<2	—	6.7	8.4	≥8.0
	LVG	<2	3.9	4.2	—	4.5
Spleen	MHA	<2	—	8.0	8.1	8.4
	LVG	<2	5.65	6.4	2.5	2.7
Brain	MHA	<2	—	5.6	6.62	—
	LVG	<2	<2	<2	<2	—

^a $\log_{10}$ PFU of virus per milliliter of blood or per gram of solid organs.

^b <2 indicates no virus at 1:100 dilution, the lowest dilution tested.

^c — indicates no data available.

PFU/ml were detected in sera of LVG hamsters receiving both virus and cyclophosphamide. Thus, in the presence of cyclophosphamide the replication of Pichinde virus and mortality in adult LVG hamsters were similar to those observed in adult MHA hamsters not treated with the drug.

Antibody response to Pichinde virus in LVG and MHA hamsters. The high virus titers and fatal outcome associated with Pichinde virus infections in MHA hamsters could represent an inability of this strain of hamster to respond to the antigens of the virus. To test this possibility, the antibody responses against two classes of viral antigens were compared between the two strains of hamsters. The major antigen detected in the complement-fixation reaction appears to be an internal component of the virion (Buchmeier and Rawls, in press). Antibodies detectable by the complement-fixation test were found in the sera of both strains of hamsters (Table 3). The mean titer of antibodies for LVG hamsters infected 10 to 13 days

FIG. 6. Effect of cyclophosphamide immunosuppression on mortality of adult LVG and MHA hamsters. The animals were intraperitoneally infected with 2.5×10^6 PFU of Pichinde virus and then either given 100 mg (MHA) or 150 mg (LVG) of cyclophosphamide per kg intraperitoneally 3 days after infection (○) or left untreated (●). There was no mortality among hamsters given the drug alone.

FIG. 5. Growth of Pichinde virus in LVG and MHA hamster macrophages cultured *in vitro*. Peritoneal exudate macrophages were harvested 3 days after stimulation with sterile mineral oil and infected with approximately 1 to 3 PFU of Pichinde virus per cell. After adsorption for 60 min at 4°C, the cells were washed and then seeded into culture tubes (2×10^6 cells/tube). Two tubes were removed at each time point, and total medium and cell-associated virus was titered. Symbols: ●, LVG macrophages; ○, MHA macrophages.

earlier with the virus was 1:53, which was slightly higher than the mean titer of 1:38 for sera from similarly infected MHA hamsters. The titers of antibody in sera of later bleedings were not significantly different between strains; however, only MHA animals that survived beyond the usual early death period were available, making their representativeness suspect.

Pichinde virus is relatively noncytotoxic, and antigens that appear on the surface of virus-infected cells may induce immunological reactions required to limit virus replication and spread. Antibodies to the surface antigens were assayed by the ability of test sera to block the binding of anti-Pichinde virus 125 I-labeled IgG to viable cells. The results are shown in Fig. 7. The means of the percentage of maximum binding of 125 I of the dilution of blocking serum are depicted as broken lines in the figure, and arrows indicate the dilution giving 50% blocking. For LVG hamsters, a titer of approximately 1:200 was recorded for LVG hamsters bled 10 to 13 days after infection (Fig. 7A); the titers increased to approximately 1:1,500 by 21 days

TABLE 3. Mean complement-fixing antibody titers in LVG and MHA hamsters at various intervals after Pichinde virus infection

Hamster strain	Day after infection	CF antibody titer ^a	
		Virus specific ^b	Anticomplementary ^c
LVG	10-13 (3) ^d	1:53 ^e	≤1:2
	21 (2)	1:256	≤1:2
	30 (5)	1:128	≤1:2
	60 (7)	1:64	≤1:2
MHA	10-13 (5)	1:38	≤1:2
	21 (2)	1:128	1:4
	30 (4)	1:128	1:21

^a Highest dilution giving 50% end point in complement-fixation (CF) test against 5 U of antigen.

^b Determined against antigen prepared from BHK-21 cells infected with Pichinde virus.

^c Fixation of complement in the absence of virus-specific antigen.

^d Number of samples tested is indicated in parentheses.

^e Geometric mean CF antibody titer.

(Fig. 7C) and dropped to 1:1,000 by 30 days (Fig. 7E). Antibodies capable of blocking ¹²⁵I-labeled IgG binding were also detected in the sera of infected MHA hamsters, but the titers were somewhat lower (Fig. 7B, D, and F). Thus, no major qualitative differences in the humoral immune responses to either virus antigen by LVG and MHA hamsters were apparent.

DISCUSSION

Different patterns of host responses to the arenaviruses have been described. LCM virus infections in mice have been most extensively studied, and many of the earlier studies have been reviewed by Hotchin (13, 14) and Lehmann-Grube (17). Although all strains of LCM virus do not produce exactly the same responses in all strains of mice, a prototype response can be constructed that can be used for comparison with other arenavirus-host interactions. Inoculation of newborn mice with LCM virus produces no apparent illness, but frequently leads to a persistent infection, whereas infection beyond the newborn period not uncommonly results in an acute fatal illness (6, 13, 14, 17). The age at inoculation is an important determinant, and the animals go from a state of relative immunological hyporesponsiveness and survival to a state of immunological responsiveness resulting in death, with the transition occurring at about 4 to 5 days of age (11). It is cell-mediated immunity and not humoral immunity that is responsible for the immunopathological changes that are associated with fatal disease

(6, 8, 13, 14, 21, 24). The development of cell-mediated immunity to the virus does not appear to influence the replication of LCM virus in the brains of mice inoculated intracerebrally (6, 13), and adult animals treated with immunosuppressants do not develop fatal illness, but may develop persistent infections (9, 13, 24).

Three other arenaviruses have been injected into their natural host under experimental conditions, and death from acute illness has not been observed, regardless of the age at infection (15, 16, 20, 27). Age at inoculation, however, does appear to influence the extent of virus replication. Tamiami virus replicates more extensively in suckling cotton rats than in adult animals, and the extent of virus replication correlated inversely with the immunological responsiveness of the host as assessed by antibody response (20). The proportion of *Calomys callosus* that produce antibody to Machupo virus also depends on the age at inoculation; persistent viremia without antibody occurred in all animals inoculated prior to 9 days of age, whereas about one half of the animals inoculated later in life produced antibodies and lost their viremia (15, 16). The role of cell-mediated immunity in limiting virus replication was not evaluated in these studies.

Members of the Tacaribe complex of viruses produce lethal infections when injected into newborn mice or hamsters; infection of adult animals normally is not associated with illness (2, 15). The pathogenesis of Tacaribe virus in mice as related to age at inoculation has been examined in some detail. Newborn animals injected intraperitoneally or intracerebrally died with low doses of virus, and 7-day-old animals died after injection of moderate doses of virus intracerebrally, whereas high doses of virus injected intracerebrally were required to kill 28-day-old animals. It is of interest that the survival time decreased with increasing age at inoculation; the mean survival time of mice inoculated at birth was 11.5 days, whereas mice inoculated at 4 to 7 days of 10 to 14 days had mean survival times of 8.5 and 6.9 days, respectively (4). Antilymphocyte serum was found to prolong the survival time (3), whereas injection of immune spleen cells decreased the survival time (4). These observations suggest that cell-mediated immunity may both limit virus replication and induce immunopathological changes. The replication of virus in newborn mice is extensive and, when cell-mediated immunity develops, sufficient immune-induced destruction is produced to kill the mice, whereas in adult mice the immune response is immediate and virus replication is limited (21). Similar conclusions can be reached from studies

FIG. 7. Blocking of ^{125}I -labeled anti-Pichinde virus IgG by sera from LVG and MHA hamsters. Each point represents blocking by serum from an individual animal, and the broken lines connect the mean at each serum dilution. The arrows indicate the point where ^{125}I binding is inhibited by 50%. LVG hamsters were bled at (A) 10 to 13 days, (C) 21 days, and (E) 30 days after infection. MHA hamsters were also bled at (B) 10 to 13 days, (D) 21 days, and (F) 30 days after infection. Points and the solid line at the bottom of (E) represent blocking of ^{125}I -labeled IgG by normal hamster sera.

of newborn mice that were thymectomized and infected with several members of the Tacaribe complex of viruses (2). Interestingly, Amapari virus responded differently and was lethal to thymectomized mice, whereas the other viruses were not.

Our data describes yet another host response to an arenavirus. Pichinde virus produced a lethal disease in newborn hamsters; however, LVG hamsters acquired resistance to a lethal infection at about 5 to 7 days of age. MHA hamsters did not develop resistance and remained susceptible as adults. The resistance of LVG hamsters could be abolished by treating the animals with the immunosuppressant cyclophosphamide. In all instances, mortality correlated with high virus titers in the blood and tissues of the animals. The correlation of death with virus titers, conversion of resistance to susceptibility of adult LVG hamsters with cy-

clophosphamide, and the rather constant survival time in relation to age at inoculation among lethally infected MHA animals suggest that death was related to virus-induced pathological changes and was not immunopathological in nature. Cell-mediated immunity appears to limit Pichinde virus replication, but does not appear to contribute significantly to disease production.

The reasons for the differences in the responses of LVG and MHA hamster to Pichinde virus are not understood. Pichinde virus did not produce a lethal infection in four other strains of inbred hamsters (data not presented). Several other arenaviruses were found not to produce a lethal disease in MHA hamsters (K. M. Johnson, personal communication). Although we believe genetic differences between the strains probably are responsible, we do not yet have data to establish this point. Genetic fac-

tors have been shown to influence the outcome of infection in a number of virus-host interactions; these have been reviewed recently by Pincus and Snyder (23). A genetic resistance of cells to replicate virus efficiently has been noted for several virus-host systems (7, 12, 27); however, we found no differences between cells from LVG and MHA hamsters in their ability to support the replication of Pichinde virus. With mouse hepatitis virus, differences in susceptibility or resistance of macrophages in tissue culture were found to correlate with susceptibility or resistance of the mice (1, 26). This does not seem to be the case with Pichinde virus since the virus grew equally well in macrophages from both strains of hamsters.

Qualitative differences in the immune response of different strains of mice to LCM virus have been related to the development of chronic immune-complex disease, and the responsiveness appeared to be histocompatibility linked (18, 22). These observations with LCM virus suggest that the genetically controlled ability of the host to respond to arenaviruses may be important in disease development. In our model there is very little information available to support or refute this possibility. Persistence of the arenaviruses, which appears to represent at least a degree of immune tolerance, is highly specific to the natural host of each virus. For example, *C. callosus* is the host of two arenaviruses; however, the geographic distribution of these two viruses is distinct. *C. callosus* from the area where Latino virus is present respond differently to Latino virus than do *C. callosus* from the area where Machupo virus is found and vice versa. In cross-breeding experiments, responsiveness of the male was passed to the offspring (28). *C. callosus* of either area produced antibodies to both viruses, but no assessment of cell-mediated immunity was made. In our study, both LVG and MHA hamsters produced antibodies to Pichinde virus, and the antibodies were to both the complement-fixing antigens and the antigens that appear on the surface of virus-infected cells. However, further studies will be required to determine whether the MHA hamsters develop a cell-mediated immune response to Pichinde virus-specific antigens.

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