Effect of Antilymphocyte Serum on Tacaribe Virus Infection in Infant Mice

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Tacaribe virus, a member of the arenavirus group, was demonstrated to have similarities in pathogenesis to the prototype virus of this group, lymphocytic choriomeningitis (LCM) virus. Treatment with antilymphocyte serum (ALS) doubled the survival time of mice neonatally infected with a lethal dose of Tacaribe virus. Twenty-one per cent of ALS-treated infected animals survived more than 30 days. All animals had recoverable virus in brains and serum for the duration of life, and there was no difference between virus growth curves of ALS-treated and control animals. No neutralizing or complement-fixing antibodies were present in infected animals at time of death, with the exception of one long-surviving ALS-treated animal. Certain differences between infections of mice with LCM and Tacaribe viruses were apparent. Tacaribe virus pathogenicity decreased with increasing mouse age, and earlier infection and a more prolonged course of ALS treatment than was needed for LCM virus were necessary to achieve increased survival. Histopathological changes observed in Tacaribe virus-infected control mice were focal choroiditis and focal cerebellar necrosis; these changes were not seen in asymptomatic infected mice during the period of transient protection by ALS.

On the basis of common physicochemical, morphological, and antigenic relationships (5, 21, 26), a new virus taxonomic group, the arenaviruses, has been established (25); viruses of this group include lymphocytic choriomeningitis (LCM), Tacaribe, Junin, Machupo, Lassa, and several other agents. Because of its biological behavior, LCM virus has long been of interest as a model for chronic viral carrier states and viral-induced immunopathogenic disease. Previous studies have demonstrated that antilymphocyte serum (ALS) not only prevented the mortality associated with LCM infection of adult mice (7, 10, 18) but also permitted the development of the viral carrier state in infant animals at an older age than was normally possible (9).

Similar biological properties and animal pathogenesis may be characteristic of all members of the arenavirus group. Like LCM virus, Machupo virus has been demonstrated to induce a chronic viral carrier state in its natural rodent host, *Calomys callosus* (13). In a further parallel, Junin virus immunopathogenicity for mice was prevented by neonatal thymectomy (30). To further examine similarities in the biological

characteristics of the arenaviruses, we have studied the effects of ALS on infection of suckling mice (SM) with Tacaribe virus, a virus initially isolated from *Artebius* bats (6), which is pathogenic for both suckling and adult mice (6) and which multiplies predominantly in the brain (8).

MATERIALS AND METHODS

Viruses. A stock of Tacaribe virus TR11573 which had undergone 21 SM brain passages and one Vero cell passage was used throughout the study. This virus stock was obtained from Adrian Chappell, Arbovirology Unit, Center for Disease Control (CDC). It titrated $10^{6.3}$ median lethal doses (LD_{50})/ml upon intracerebral (ic) inoculation of 1- to 3-day-old SM. The LCM virus used (Armstrong ATCC E-350 strain mouse brain stock) titrated $10^{7.1}$ LD_{50} /ml upon ic inoculation of 1-week-old mice.

Animals. Swiss mice of the ICR strain, free from observable intercurrent infections, were used for all studies.

Antilymphocyte serum. Horse antimouse thymocyte serum (HAMT; lot no. 9716) was obtained from Robbin Laboratories, Chapel Hill, N.C. It was determined by them to be effective in suppressing the rejection by C57BL10/ScSn mice of a 3-methylcholanthrene induced sarcoma, differing at the H-2 locus from its donor host, a B10D2 mouse. The HAMT administered intraperitoneally (ip) to adult

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mice in our laboratory depressed the peripheral mononuclear count 70 to 80%. It was also effective, as shown below, in preventing signs of LCM infection of 1-week-old mice. This assay has proved reliable in our laboratory as a measure of cellular immune suppression (9). Neither HMT nor normal horse serum (NHS; Grand Island Biological Co., Grand Island, N.Y.) was toxic upon repeated administration: neonatal mice given 0.9 ml by the ip route in 13 divided doses over 30 days from birth were not runted or otherwise clinically distinguishable from uninoculated controls during development or at 8 weeks of age. During the period of serum administration to this uninfected control group, none of 15 animals given HMT and two of 19 given NHS died. The frequency of HMT administration in relation to time of virus inoculation varied in different experiments, as indicated below, but HMT was always given ip in 0.05-ml doses.

Virus titrations. Titrations of LCM and Tacaribe viruses were performed in SM 1 to 3 days old; 0.02 ml of virus dilutions was given ic. The LD_{50} end points were determined by the method of Kärber (14). No difficulty was encountered in determining end points for Tacaribe virus in mouse titrations.

Titrations of Tacaribe virus in serum and brain tissue of infected animals were performed on pooled material from three animals. Serial dilutions were prepared in Eagles minimal essential growth medium containing 10% newborn calf serum.

Complement-fixation tests. Complement-fixation (CF) tests on serum were performed using the LBCF microtechnique against mouse brain antigen extracted by Veronal-buffered diluent (4).

Histopathology. Sections were prepared from organs of animals sacrificed at regular intervals after Tacaribe virus inoculation. The animals were perfused with saline and then with 10% buffered Formalin fixative, and organs were stained with hematoxylin and eosin.

RESULTS

Tacaribe virus infection in normal mice. Neonatal mice inoculated ic within 24 hr of birth with 10, 100, or 1,000 LD_{50} of Tacaribe virus developed clinical signs of infection by days 9 to 11. Early signs included ruffled fur, edema-

TABLE 1. Titer^a of a Tacaribe virus stock in different-aged mice inoculated by the intracranial (ic) and intraperitoneal (ip) routes

Age (days)	ic Route	ip Route
0 ^b	4.6	4.3
7	4.6	<1.0
28	0.5	0

^a $\log_{10} LD_{50}/0.02$ ml. All animals were observed for 25 days.

^b Inoculated within 18 hr of birth.

FIG. 1. Survival of newborn mice treated with horse antimouse thymocyte serum (HMT) or normal horse serum (NHS) on days 7, 4, and 1 before inoculation with 1,000 LD_{50} of LCM virus (A) or Tacaribe virus (B).

tous facies, and generalized tremor with progression to ataxia, tonic seizures, and occasional paralysis of the hind extremities. Death occurred 1 to 3 days after the onset of illness. The virus was equally pathogenic for newborn mice by either the ic or the ip route but killed older animals only by the ic route (Table 1). There was no apparent difference in susceptibility of newborn and 7-day-old mice inoculated by the ic route. In adult animals, 10,000 ic inoculated SM LD_{50} were uniformly fatal within 3 to 5 days, but lesser amounts failed to evoke symptoms.

Tacaribe virus infection in HMT-treated mice. Twenty-six mice were inoculated ip on days 0, 3, and 6 of life with 0.05 ml of HMT, and 29 others were similarly inoculated with 0.05 ml of NHS as controls. All were then inoculated ic on day 7 with 1,000 LD_{50} of Tacaribe virus. In parallel series, 28 mice which received HMT and 20 which received NHS at the same ages and schedule as above were inoculated ic on day 7 with 1,000 LD_{50} of LCM virus.

The HMT treatment schedule prevented mortality of LCM-infected animals; those which received NHS suffered a 95% mortality (Fig. 1A). On the other hand, in the Tacaribe-infected mice the HMT treatment did not prevent mortality but did delay the first mouse death by 2 days. The 50% survival time was also ex-

tended 2 days (Fig. 1B). This delay in mortality was statistically significant ($P \ll 0.01$) when analyzed by Kolmogorov-Smirnov two sample tests (27) kindly performed by Elmer Hall.

Mice which were inoculated with only 10 LD_{50} of Tacaribe virus after similarly scheduled treatment with HMT showed no differences in death patterns from the NHS controls; however, the death pattern and delay in death after inoculation with 100 LD_{50} were comparable to those seen after inoculation with 1,000 LD_{50} .

To determine whether increasing the duration and total amount of HMT treatment would further prolong the survival of Tacaribe-infected animals, 38 mice were given 0.05 ml of HMT on days 0, 3, and 6 of life; they were inoculated with 1,000 LD_{50} of Tacaribe virus on day 7 and were given additional 0.05-ml amounts of HMT on days 9, 12, and 15. Forty-one controls received NHS instead of HMT. The additional HMT treatment did not further prolong survival; a delay in mortality similar to that caused by the shorter HMT treatment schedule was observed.

Further decrease in the mortality caused by Tacaribe infection was achieved by HMT treatment when the mice were infected on the day of birth. A group of 36 newborn mice were treated with 0.05 ml of HMT on days 0, 2, 4, 6, 8, 10, 12, 14, and 16; 60 control mice received NHS. Both mouse groups were inoculated with 100 LD_{50} of Tacaribe virus 4 hr after the first HMT or NHS treatment. An unequivocal prolongation in survival of the HMT-treated group resulted (Fig. 2A). There was a 10- to 12-day delay of the first death, of the 50% survival time, and of the last virus death. The weights of HMT-treated animals were not significantly different from those of NHS controls. Extending HMT treatment of surviving animals beyond day 16 (0.1 ml of HMT every 3 days to day 30) resulted in a similar overall effect (Fig. 2B) except that four out of 21 mice survived more than 30 days. Of these four survivors, one (S1) died on day 35 and had a large subhepatic abscess on autopsy; a second (S2) died on day 37 and had a peritoneal abscess; and a third (S3) was found cannibalized on day 39. The fourth (S4) was sacrificed on day 70; it showed no clinical evidence of infection and was of normal weight.

Virus titrations. Littermates of the mouse group which received virus on day 0 and HMT or NHS for 30 days (Fig. 2B) were sacrificed at 2-day intervals to day 12, and littermates of the HMT group were sacrificed on day 19 for virus titration. Tacaribe virus titers in serum and brain of both the NHS- and HMT-treated

FIG. 2. Survival of mice treated with horse anti-mouse thymocyte serum (HMT) or normal horse serum (NHS) for 16 days (A) or 30 days (B) after inoculation on the day of birth with 100 LD_{50} of Tacaribe virus.

groups were similar at each sample time (Fig. 3). By day 19 serum and brain titers in the HMT animals had declined slightly. Of the HMT-treated animals surviving more than 30 days, S1 had $10^{1.5}$ LD_{50} /0.02 g of brain at death, S2 had $10^{1.5}$ LD_{50} , and S4 had $10^{0.6}$ LD_{50} . No virus was recoverable from the serum of S4. The virus isolated from these animals was confirmed as Tacaribe by CF test against a reference hyper-immune serum.

Antibody studies. No CF antibody (titer $<1:4$) for Tacaribe virus was demonstrable on day 12 or day 19 in a serum pool from animals inoculated with Tacaribe virus on day 0 and treated with HMT for 30 days (Fig. 2B) or on day 12 from a serum pool of sick NHS-treated animals. Animals S1 and S2 also had no CF antibody (titer $<1:20$). Animal S4, however, had CF antibody at a titer of $>1:512$ at the optimum antigen dilution. On day 19, HMT-treated survivors from the group in Fig. 2B probably had no effective neutralizing antibody, since their serum, inoculated into other mice, had $10^{3.1}$ LD_{50} /0.02 ml of infectious Tacaribe virus.

Histopathology. Two histopathologic lesions were observed in the brains of mice treated with NHS and sacrificed 12 to 14 days after infection

FIG. 3. Tacaribe virus in brain (A) and serum (B) of mice inoculated with 100 LD₅₀ of Tacaribe virus on day 0 of life and then treated for 30 days with horse antimouse thymocyte serum (HAMT, solid line) or normal horse serum (NHS, dotted line), see Fig. 2B.

with 100 LD₅₀ of Tacaribe virus: (i) a severe choroiditis (similar to that produced by LCM virus in mice but with little ependymitis or leptomeningitis characteristic of LCM virus infection) and (ii) a focal necrotic lesion of the cerebellar cortex, which destroyed some folia but left most folia intact. Neither of these lesions was seen in HAMT-treated mice, sacrificed up to 19 days after infection. No pathologic alterations were noted in bone marrow, spleen, liver, kidney, heart, lung, bone, or gastrointestinal tract of infected animals from either group.

DISCUSSION

Two biological characteristics of the arenaviruses, mentioned in the definition of the group but not considered an integral part of it (25), are (i) production of a chronic virus carrier state in the rodent species which is the natural host and (ii) a pathogenicity which varies according to the integrity of the thymus-dependent lymphoid system. These host-virus interactions have been described in detail for LCM (the prototype virus) but are less well established for other members of the arenavirus group.

Persistent infection. A salient aspect of LCM virus infection is persistent viremia without neutralizing antibody (11, 28). Infections of this kind can be induced by vertical transmission of the virus in utero (28), infection within 24 hr of birth (11), or infection of neonatally thymectomized (24) or ALS-treated (9) animals.

Likewise, Tacaribe virus-infected mice, treated with ALS, became virus carriers for the duration of life. The brain of healthy-appearing animals on the 19th day of infection contained almost 10⁷ LD₅₀ of virus/0.02 g, and serum had 10^{1.8} LD₅₀ of virus/0.02 ml. Animals surviving more than 30 days all had measurable virus in the brain, although the titers had decreased. In spite of persistent viremia, no neutralizing antibody was evident. CF antibodies were present in only one animal, S4, which survived with recoverable virus and had a CF antibody titer of ≥1:512 upon sacrifice on day 70 of infection.

There were, however, quantitative differences in the effect of ALS on Tacaribe and LCM infections. Thus, a course of ALS treatment resulted in only a short, although significant, prolongation of survival of mice inoculated with Tacaribe virus on day 7 of life. Animals given a similar course of ALS and inoculated on day 7 of life with LCM virus survived for more than 60 days (Fig. 1) and have been shown to be chronic virus carriers (9).

Further evidence for the common biological behavior of arenaviruses comes from studies of Machupo, Junin, and Lassa viruses. Machupo virus can be recovered for up to 285 days after ip infection of the hamster (12) and for at least 1 yr in blood and urine after ip infection of infant and some adult *C. callosus* (13). The infant *Calomys* show no evidence of disease or neutralizing antibody and in nature the virus has been repeatedly isolated from this species (13). Junin virus has been detected in the infant hamster 45 days after infection (1). Mice, thymectomized at birth, were protected from a normally lethal infection with Junin virus after ic inoculation (29); the virus was recoverable from the brains of surviving mice after 50 days but no CF antibodies were ever detected. After nonfatal infection of adult mice with Lassa virus, prolonged viremia has been observed (2).

Immunological mechanisms of disease production. After infection with LCM virus, both the fatal choriomeningitis, which results from ic inoculation of adult mice, and the glomerulonephritis are considered to be mediated by immunological mechanisms (11, 22, 23, 29).

The pathogenesis of Tacaribe virus infection is apparently similar to that of LCM virus infection. In both cases it is likely that pathological

effects result from activation of cellular immune processes against the virus. ALS, which has as a primary effect suppression of cellular immunity (15, 16), delayed the outcome of a usually fatal Tacaribe virus infection. Continuous treatment with ALS doubled the survival of mice infected with Tacaribe virus on the day of birth (Fig. 2). The multiplication of Tacaribe virus was not suppressed in this group; virus growth curves in serum and brain of control and ALS-treated groups were similar (Fig. 3). In the infected groups there was no correlation between virus titers and pathogenicity. Even in animals receiving virus only, titers of greater than 10^7 LD₅₀/0.02 g of brain were found 4 to 6 days before signs of illness.

Certain differences between LCM and Tacaribe virus infections in the mouse were apparent and probably resulted from the differing host-virus relationship. Tacaribe virus in small amounts was lethal to neonatal mice; larger amounts were necessary to kill adult animals (Table 1). In contrast, the pathogenicity of LCM virus increases with increasing age (11).

In Tacaribe virus-infected mice, the choroiditis was quite similar to that produced by LCM virus and probably contributed to mortality, but the inflammatory response was focal and did not involve the ependyma, meninges, or brain parenchyma. The cerebellar lesion produced in mice by Tacaribe virus has not been reported in mice infected with LCM virus. However, LCM virus has recently been reported (20) to produce cerebellar necrosis in neonatally infected rats. Furthermore, Tamiami, another arenavirus, has been shown to produce a similar lesion in mice (3).

A complex balance of virus-host interactions seems to determine the outcome of arenavirus infection. Absence of both cellular and humoral immune reactivity produces a state of prolonged survival and persistent viral carriage in the newborn mouse infected with LCM (11, 28) or in *C. callosus* infected with Machupo virus (13). Such an animal probably represents a reservoir host. Pathological changes, which develop most rapidly in the unnatural host, result from an intense cellular immune response (10, 11, 24) and the development of complement-mediated immune complex disease (22, 23). Pathogenicity can be altered by the use of immunosuppressant measures as exemplified by ALS treatment for Tacaribe virus infection and thymectomy for Junin virus infection of mice (30). Pathogenicity can also be influenced by immunological maturity of the host (11) and possibly the degree of virus-mediated immune suppression (19).

Overcoming an arenavirus infection probably depends upon the strength of the neutralizing antibody response (29), upon cell-mediated viral elimination (17), and upon undefined host genetic defenses (11, 22, 29).

Arenavirus infection of man is an unnatural virus-host relationship, often characterized by infections which may be lethal (Lassa, Machupo, Junin) or of limited duration (LCM). For this reason Tacaribe virus infection of mice may be a useful model for study of the pathogenesis of human arenavirus infection.

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