Cross-Protection in Nonhuman Primates Against Argentine Hemorrhagic Fever

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The susceptibility of the marmoset Callithrix jacchus to Tacaribe virus infection was investigated to perform cross-protection studies between Junin and Tacaribe viruses. Five marmosets inoculated with Tacaribe virus failed to show any signs of disease, any alterations in erythrocyte, leukocyte, reticulocyte, and platelet counts or any changes in hematocrit or hemoglobin values. No Tacaribe virus could be recovered from blood at any time postinfection. Anti-Tacaribe neutralizing antibodies appeared 3 weeks postinfection. The five Tacaribe-infected marmosets and four noninfected controls were challenged with the pathogenic strain of Junin virus on day 60 post-Tacaribe infection. The former group showed no signs of disease, no viremia, and no challenge virus replication, whereas the control group exhibited the typical symptoms of Argentine hemorrhagic fever, high viremia, and viral titers in organs. Soon after challenge, the Tacaribe-protected marmosets synthesized neutralizing antibodies against Junin virus. These results indicate that the marmoset C. jacchus can be considered an experimental model for protection studies with arenaviruses and that the Tacaribe virus could be considered as a potential vaccine against Junin virus.

Junin virus, a member of Arenaviridae, induces a severe, systemic disease in humans known as Argentine hemorrhagic fever (14). Several animal species such as guinea pigs, hamsters, mice, and rats are also susceptible to Junin virus infection, developing hemorrhagic or neurological diseases or both (12, 20). Guinea pigs infected with pathogenic strains of Junin virus develop a fatal disease akin to human AHF (7, 20). Therefore, up to now, the development of a lethal disease in guinea pigs is the best virulence marker for Junin virus strains (1, 8). In fact, it is guinea pigs in which protection experiments against pathogenic strains of Junin virus can be conducted (8). At the same time, guinea pigs are the only animals in which cross-protection experiments against Junin have been performed (4, 19).

Recent research carried out in our laboratory shows that Junin virus causes a lethal disease in the marmoset Callithrix jacchus. The infection is characterized by pronounced and long-lasting viremia, leukopenia, anemia, and thrombocytopenia, as well as hemorrhagic or neurological symptoms or both (9, 17).

Previous cross-protection studies with the antigenically related Tacaribe virus have shown that this virus fails to induce an apparent disease in guinea pigs. However, the animals are thereafter protected against challenge with lethal doses of Junin virus while developing anti-Tacaribe and anti-Junin antibodies (3, 16, 19). Once the susceptibility of C. jacchus to Junin virus infection was established, it was decided to determine whether the infection of this marmoset with Tacaribe virus had any pathogenic effect and to see whether they became protected against a later challenge with Junin virus.

The results reported herein demonstrate that Tacaribe virus failed to induce any disease in marmosets and that, at the same time, it protected them from subsequent challenge with lethal doses of Junin virus.

MATERIALS AND METHODS

Primates. Nine wild adult marmosets (C. jacchus) weighing 280 to 330 g were used throughout the experiment. These animals were obtained from CAPRIM (Argentine Primate Center) where they were quarantined for at least 6 months before being sent to our laboratory.

Marmosets were caged in an isolated laboratory at 24 to 27°C and above 50% humidity. They were fed with an aqueous mixture of Nestum (Nestlé, S.A.), Gevrail proteina (Lederal Cyanamid, Buenos Aires, Argentina, S.A.), powdered milk, apples, and ba-
This diet was supplemented with vitamins and minerals. Water was provided ad libitum.

**Virus.** Tacaribe virus TRVL 11 573 strain provided by Charles J. Pfau, Rensselaer Polytechnical Institute, Troy, N.Y., after two mouse brain passages in our laboratory with a titer of 10^{6.5} 50% tissue culture infectious doses (TCID_{50}) per ml in Vero cells were used.

Junin virus, the prototype pathogenic XJ strain, isolated in our laboratory from human blood in 1958, with 27 subsequent passages in guinea pigs and 29 passages in mice was used. This stock had a titer of 10^{7.7} TCID_{50}/ml in Vero cells.

Viral stocks were both 10% homogenates of infected suckling mouse brain in Hanks medium with 5% inactivated calf serum.

**Cell cultures.** Vero cells cultured in tubes 48 to 72 h before inoculation were kept in Eagle minimal essential medium supplemented with 10% heat-inactivated calf serum.

**Hematology.** Erythrocyte, platelet, leukocyte, and reticulocyte counts, as well as hematocrit and hemoglobin concentration, were performed by the usual methods.

**Virus titration and neutralization test.** Brain virus titers were determined by cytopathic effect on Vero cells. Blood titers were determined by intracerebral inoculation of new born mice. The TCID_{50} and 50% lethal dose were calculated by the Reed-Muench method.

Tests for neutralizing antibodies were performed on Vero cell monolayers, using the constant virus-varying serum dilution technique. Antibody titers were expressed as the reciprocal of the highest dilution of serum which entirely inhibited the cytopathic effect of virus.

**Inoculation procedures.** C. jacchus were divided into two groups of five and four animals. Five marmosets were inoculated intramuscularly with 10^{6} TCID_{50} of Tacaribe virus in a total volume of 0.2 ml, and four C. jacchus were maintained as uninoculated controls.

At day 60 after infection with Tacaribe virus, the nine marmosets of both groups were challenged with 1,000 TCID_{50} of Junin virus by intramuscular route.

Marmosets were observed daily for clinical symptoms, and body weight was recorded.

Blood samples were obtained from the femoral vein of both groups before infection and at 7, 14, 21, 28, 45, and 60 days after infection with Tacaribe virus and at 7, 14, 21, 28, 96, 165, 250, and 285 days after challenge with Junin virus, to perform hematological studies and assays of viremia and serum neutralizing antibodies. To compare the spread and replication of the challenge virus in monkeys with or without previous Tacaribe virus infection, two marmosets (one from each group) were killed at 18 and 21 days after inoculation with Junin virus. Brain, kidney, lung, spleen node, liver, bone marrow, salivary and adrenal glands, thymus, heart, and pancreas were aseptically removed and kept at -70°C until viral titration in Vero cells was carried out. In all animals complete necropsies were performed immediately after death.

**RESULTS**

The weight curves comparing the non-inoculated control group and the Tacaribe-infected group were roughly parallel (Fig. 1), offering negligible differences up to 60 days postinfection (p.i.) when both groups were challenged with Junin virus. From that moment onward, control animals presented the loss of weight characteristic of Junin virus-infected marmosets (18), whereas the weight of those previously infected with Tacaribe virus remained unaltered. Besides losing weight, marmosets infected with Junin virus alone developed the symptoms already described for Argentine hemorrhagic fever in C. jacchus (17), including anorexia, lassitude, dehydration, gum bleeding, hemorrhages, anemia, thrombocytopenia, and leukopenia (Fig. 2 and 3).

Neither clinical alterations nor changes in
behavior or feeding habits were observed in marmosets infected with Tacaribe virus even after Junin virus challenge. In addition, there were no hematological modifications in erythrocyte counts or in hematocrit, hemoglobin, or reticulocyte values (Fig. 2). Neither were changes detected in platelets or leukocytes subsequent to double infection (Fig. 3).

Positive viremia developed only in control marmosets infected with Junin virus alone (Fig. 4). The onset of detectable viremia was at day 7 p.i., reaching a peak of 6 log at day 14 p.i. High viremia levels persisted until death. No virus could be detected in the blood of marmosets which had previously received Tacaribe virus.

Two marmosets infected only with Junin virus died at 21 and 24 days p.i., and the other two were killed at 18 and 21 days p.i., when they were obviously ill. Two out of five healthy marmosets infected with both viruses were also killed on days 18 and 21 after Junin infection to determine viral spread and replication. High titers of Junin virus were found in blood and in all organs assayed in C. jacchus infected with Junin virus alone (Fig. 5), whereas no virus could be isolated from any organ of monkeys previously infected with Tacaribe virus.

Serological studies showed that by day 21 after Tacaribe infection, homologous antibodies (against Tacaribe virus) appeared in the sera of infected animals, with values rising steadily up to day 60, when the marmosets were challenged with Junin virus. Thereafter, an increase in the antibody level occurred (Fig. 6). Heterologous antibodies (against Junin virus) were not found up to the time of challenge in Tacaribe-infected marmosets (Fig. 6), but upon inoculation with Junin virus, significant amounts of anti-Junin antibodies were rapidly synthesized and remained constant until day 225 after Tacaribe infection, after which both anti-Tacaribe and anti-Junin declined slowly till day 345, when the experiment was brought to an end.

DISCUSSION

The marmoset C. jacchus, known to be highly susceptible to Junin virus (9, 17, 18) failed to exhibit any clinical symptoms, viremia, or hematological alterations, when infected with 10⁵ TCID₅₀ of Tacaribe virus. In fact, no changes

FIG. 2. Erythrocyte, hemoglobin, hematocrit, and reticulocyte values in Tacaribe virus (T)-infected C. jacchus challenged with 1,000 TCID₅₀ of Junin virus (J). The table at the bottom indicates the standard errors (SE) of the mean at different days p.i.
were detected in hemoglobin and hematocrit values or reticulocyte, platelet, leukocyte, and erythrocyte counts.

It was possible to demonstrate that Tacaribe-infected *C. jacchus* were fully protected against high lethal doses of a pathogenic strain of Junin virus. Furthermore, in the protected marmosets, viremia was absent, and spread and replication of the challenge virus were inhibited.

It is evident that Tacaribe-infected marmosets were protected by mechanisms that inhibited viral replication, viremia, and spread of Junin virus challenge. Passive immunization studies performed in guinea pigs and humans infected with Junin virus have demonstrated the important role of neutralizing antibodies in the protection against the disease and death induced by this virus (11, 21). Humoral neutralizing heterologous (anti-Junin) antibodies were detected in marmosets at 14 days after Junin virus infection. In this case, prechallenge antibody levels to Junin virus were not correlated with levels of protection. The absence of anti-Junin antibodies in Tacaribe-infected monkeys up to the time of challenge contrasts with the results obtained in guinea pigs infected with Tacaribe virus, where late anti-Junin antibodies (45 days p.i.) could be detected before challenge (2). In these animals the protective effect against Junin virus was also demonstrated at early stages (3 to 10 days p.i.) before detection of any humoral neutralizing antibodies. It has been postulated that viral
interference or cellular immunity (or both) may play an important role in the early protection against Junin observed in guinea pigs (2, 6). It is also suggested that cell-mediated immunity plays a major role in heterologous protection among togaviruses (5, 10). The role of cellular immunity was not studied; however, it is possible that protection in marmosets may have been effected by a cell-mediated immune response acting together with low levels of specific anti-Junin antibodies, not detectable by the techniques used in this experiment, and an anamnestic-like immune response after challenge.

After Junin virus infection of Tacaribe-infected marmosets there was an early response of heterologous antibodies, probably elicited by antigens common to both the immunizing and challenge virus. Using conventional isolation methods (Vero cells and intracerebral inoculation of suckling mice) infectious Junin virus was not found in organs of Tacaribe-protected marmosets challenged with Junin virus, in contrast to the high levels found in blood and organs of nonprotected controls. It is possible that Junin virus replicates only to a limited extent, too low to be detected by the techniques used in this experiment, but enough to elicit an anamnestic-like immune response to Junin virus, as has already been suggested for the guinea pig-Tacaribe system (2). Up to now, no exhaustive search for infectious Junin virus has been undertaken. However, cocultivation with Vero cells, explant cultures, and immunofluorescent studies are in progress to detect replication or persistence (or both) of virus, both in challenged and unchal-
lenged Tacaribe-infected animals. Pathological studies are also underway with special stress on central nervous system involvement.

Tacaribe virus was isolated from bats in Trinidad between March 1956 and December 1958 (13). Since that time further isolation attempts proved unsuccessful. One of the main problems encountered with the use of live attenuated viral vaccines is the back-mutation to their original virulence. It is believed that this reversion to virulence would be most unlikely with the use of an heterologous virus originally nonpathogenic for humans, as would be the case for Tacaribe virus. Epidemiological surveys in Trinidad failed to demonstrate evidence of natural Tacaribe virus infections in humans (13). One clinical laboratory-acquired infection (with fever, headache, muscle pains, and seroconversion to Tacaribe virus) has been mentioned in a research worker handling this virus (D. H. L. Bishop and J. Casals, personal communications). An apparent infection has also been reported (15).

All these data suggest that after more exhaustive studies in primates, only experiments in humans will be able to determine whether Tacaribe virus infection is capable of protecting humans against Argentine hemorrhagic fever safely.

Some preliminary results from our laboratory with the XJCl3 strain of Junin virus, attenuated for guinea pig and humans, showed that it also proved nonpathogenic for the marmoset C. jacchus. Taking this fact into account, together with the results presented in this paper, it is suggested that this marmoset may be used as an experimental model to determine the pathogenic effect of different strains of Junin virus, as well as the virulence of other arenaviruses, by means of its ability to become infected, to develop viremia, and to acquire the disease, whether in a mild, severe, or lethal form. C. jacchus may also become the best animal model for future experiments on protection against Argentine hemorrhagic fever and for testing the efficacy and safety of experimental vaccines before clinical trials in humans.

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LITERATURE CITED