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NASAL AND INTRATHALAMIC INOCULATIONS OF PRIMATES WITH TACARIBE VIRUS: PROTECTION AGAINST ARGENTINE HEMORRHAGIC FEVER AND ABSENCE OF NEUROVIRULENCE

S. R. SAMOILOVICH[1], *J. PECCI SAAVEDRA[2]), M. J. FRIGERIO[2]), M. C. WEISSENBACHER[2])

Chair of Microbiology, Parasitology and Immunology and *Chair of Histology, Cytology and Embryology, Faculty of Medicine, University of Buenos Aires, 1121 Buenos Aires, Argentina

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Summary. — *Callithrix jacchus* marmosets were inoculated by different routes with two stocks of Tacaribe virus, one from suckling mouse brain and another from human diploid MRC5 cells. All 12 primates inoculated by nasal route developed neutralizing serum antibodies without any clinical signs. All 6 primates receiving the mouse brain-Tacaribe virus were protected against lethal challenge with pathogenic XJ strain of Junin virus, while protection was also conferred in 4 out of 6 primates receiving the diploid cell-Tacaribe virus stock. Intramuscular (i.m.) inoculation also elicited antibodies and conferred protection to 4 primates receiving the diploid cell-virus stock. Intrathalamic (i.t.) inoculation of mouse brain-virus stock caused no clinical signs or histopathologic changes in groups of 3 primates each examined on days 33 and 90 post-infection (p.i.). All primates developed antibody response, but no virus could be detected in their brain. Thus, Tacaribe virus proved harmless and immunogenic in *Callithrix jacchus* and protected most marmosets against challenge with the lethal XJ strain of Junin virus.

Key words: Tacaribe virus; Argentine Hemorrhagic Fever; neurovirulence; primates

Introduction

Tacaribe virus protects guinea pigs against the antigenically related Junin virus, the etiological agent of Argentine Hemorrhagic Fever (AHF). This protection was observed using i.m. or nasal inoculation of Tacaribe virus stocks from either mouse brain (Weissenbacher *et al.*, 1975) or diploid cells (Damonte *et al.*, 1981).

The marmoset *Callithrix jacchus* reproduces many features of the human disease when inoculated with the XJ pathogenic strain of Junin virus: leukopenia, thrombocytopenia and viremia, as well as hemorrhagic and

1) Fellow of the Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET).
2) Member of the Research Career (CONICET).

neurologic manifestations (Weissenbacher *et al.*, 1980; Frigerio *et al.*, 1982). In the primates, as in the guinea pig (Weissenbacher *et al.*, 1975) Junin virus infection causes 100% mortality. This fact, together with its phylogenic relatedness to man, makes the *C. jacchus* an adequate model to test experimental AHF vaccines. Thus, the Junin $XJCl_3$ attenuated strain and Tacaribe live viruses have been shown to induce protection in *C. jacchus* inoculated by i.m. route (Avila *et al.*, unpublished data; Weissenbacher *et al.*, 1982).

When testing an experimental vaccine in animals many properties must be studied in order to choose the best route of administration, the vaccine dose and replication substrate. Regarding the inoculum safety, high doses of virus and the routes of inoculation rendering animals susceptible to virus infection must be assayed. Due to the risk of virus reaching the central nervous system (CNS) by mucosal, neural or blood routes with subsequent acute or chronic disease, it is essential to evaluate the neurovirulence of live viral vaccines in primates. The attenuated strains of Junin virus $XJCl_3$ and XJo have low mortality for guinea pigs when administered by i.m. route, but are more pathogenic and lethal by intracerebral (i.c.) route (Boxaca *et al.*, 1982). $XJCl_3$ strain also proved pathogenic when inoculated i.c. in the Cebus monkey sp. (Carballal, 1982).

This paper reports the results of nasal, i.m. and i.t. inoculations of Tacaribe virus in order to determine the immunogenicity and safety of Tacaribe virus administration in primates. Two stocks of Tacaribe virus were used: one from suckling mouse brain, for nasal and i.t. inoculations, and the other from human diploid MRC5 cells for nasal and i.m. trials.

Materials and Methods

Viruses. Tacaribe virus strain TRVL 11573: The mouse brain stock was prepared in 2-day-old mice inoculated i.c. with 0.02 ml of viral suspension and killed 7 days later. Brains were homogenized and suspended in Hank's medium with 5% inactivated calf serum. After 1 hr centrifugation at 10,000 g, the supernatant was removed and kept at −70 °C. The stock virus titer was $10^{5.7}$ $TCID_{50}$/ml. The diploid cell stock was prepared by cloning the mouse brain stock twice in Vero cells; supernatant virus was purified by ultracentrifugation and inoculated in human MRC5 cells (passage 30) at a multiplicity of infection of 0.5. At day 5 p.i., the cells were frozen and thawed twice and then centrifuged. Supernatant was kept as a stock at −70 °C, its titer being $10^{5.9}$ $TCID_{50}$/ml.

Junin virus prototype pathogenic strain XJ: The stock was prepared as described for Tacaribe-mouse brain virus stock. For challenge of the primates, 1000 $TCID_{50}$ of the virus was used by i.m. route. This inoculum proved uniformly lethal for *Callithrix jacchus*, guinea pigs and mice used as controls.

Animals. Callithrix jacchus marmosets were caught and quarantined at the Argentine Primate Center (CAPRIM) for 6 months to rule out any disease. Tuberculosis, ectoparasites, helminthiasis and Chagas disease were specially investigated. 24 adult *Callithrix jacchus* weighing 270−350 g were housed in our laboratory as previously described (Weissenbacher *et al.*, 1980). The primates were screened for anti-Tacaribe and anti-Junin antibodies prior to each experiment, with uniformly negative results, They were observed daily, and weighed twice a week. In animals found dead, macroscopic organ examination and viral isolation were performed.

Neutralization test. Serum antibodies were assayed by the constant virus-varying serum technique on Vero cell monolayers grown in test tubes. Neutralizing antibody titers were expressed as the reciprocal of the highest dilution of serum which inhibited cytopathic effect of 100 $TCID_{50}$ of Junin $XJCl_3$ or Tacaribe viruses in 50% of the tubes.

Intrathalamic inoculation. Primates were anaesthetised with ketamine chlorhydrate (25 mg/kg body weight, intramuscular) and fixed in a stereotaxic device (La Precition Cinematographic

Francaise, mod. A, modified). Two horizontal coordinates corresponding to the right thalamus were located following an atlas of the marmosets CNS as previously described (Pecci Saavedra, 1969). At this point, a skin incision exposing the parietal bone was made, a vertical orifice drilled and the needle of a microsyringe (Hamilton Microliter 705—N) was inserted 9 mm into brain to inoculate 20 µl of virus. After 10 min the needle was removed, the orifice was packed with Gelfoam (Upjohn, U.S.A.) and the skin sutured.

Experimental infection of primates. Nasal inoculation: six primates received 0.1 ml of mouse brain-Tacaribe virus in each nostril, with a total dose of $10^{5.2}$ $TCID_{50}$ for each monkey. By day 53 p.i., the primates were bled for antibody detection and challenged with Junin virus. Six primates received a similar inoculum of diploid cell-Tacaribe virus. By day 82 p.i., these primates were bled for antibodies and challenged with Junin virus. I.m. inoculation: Four primates received 0.2 ml of diploid cell Tacaribe virus by i.c. route, the dose being $10^{5.2}$ $TCID_{50}$. These primates were bled for antibodies and challenged with Junin virus 82 days later. I.t. inoculation: six primates were inoculated as previously described with 10^4 $TCID_{50}$ of mouse brain-Tacaribe virus. Three animals were killed at 33 days and 3 at 90 days p.i. Two primates were inoculated with diluent to serve as controls, and were killed at 10 and 33 days, respectively. Histologic examination was performed in all 8 primates and viral isolation was attempted from brain by suckling mouse inoculation.

Histologic studies. In a group of primates killed after Tacaribe inoculation, samples of the following organs were taken and processed for hematoxylin-eosin staining: CNS, liver, heart, spleen, lungs, pancreas, kidneys and lymph nodes. Special attention was paid to each individual brain, at least 10 slices being examined.

Virus titration. Organ samples were processed as described for mouse brain stocks. Ten-fold dilutions of supernatants were inoculated in Vero cells monolayer or i.c. into suckling Rockland mice. Cytopathic effect or mortality were registered and tissue culture infecting dose 50 ($TCID_{50}$) or lethal dose 50 (LD_{50}) calculated by the Reed-Muench method.

Results

Intranasal inoculation of primates

From the 12 primates reciving Tacaribe virus (either the mouse brain or the diploid cell stock) by nasal route none showed clinical symptoms, behavioural alterations or weight loss as a result of disease. The 6 monkeys receiving the mouse brain-Tacaribe virus stock had anti-Tacaribe antibodies by day 53, titers being higher than 40. In 3 of them tested, no heterologous anti-Junin antibodies were detected. All 6 primates proved resistant, when challenged with pathogenic Junin virus. After a 2-month observation period asymptomatic monkeys were killed and autopsies showed no macroscopic organ alteration. In all of them serum anti-Junin antibody titer was higher than 40 (see Table 1).

The 6 animals receiving diploid cell-Tacaribe virus stock had anti-Tacaribe antibodies by day 82, titers ranging from 40 to over 320. Three of these marmosets were tested for heterologous antibodies with negative results.

When these 6 primates were challenged with Junin virus, two died at 20 and 22 days. The first dead animal, with pre-challenge Tacaribe antibody titer of 40, had Junin virus in lymph node and spleen (both titers $10^{3.5}$ $TCID_{50}$/g), though not in lungs. From the other marmoset found dead, with an antibody titer of 112 prior to challenge, Junin virus was found in lymph nodes ($10^{3.1}$ $TCID_{50}$/g) but not in spleen or lungs. In the animals surviving challenge, no lesions were found at autopsy and no virus was detected in their organs.

Table 1. Antibody titers* in C. jacchus receiving Tacaribe virus by different routes and their survival after challenge with Junin virus.

Route of inoculation	Tacaribe virus stock	Virus dose	Challenge on day:	Antibody titers on day:	Anti-Taribe titer**:	Anti-Junin titer:
nasal	mouse brain	$10^{5.2}$	53	53	>40 >40 >40 >40 >40 >40	<10 <10 <10
nasal	mouse brain	$10^{5.2}$	53	113		>40 >40
nasal	diploid cells	$10^{5.2}$	82	82	40* 112* >160 >160 320 >320	<20 <20 <20
i. m.	diploid cells	$10^{5.2}$	82	82	>160 >160 >112 120	<20 <20
i.t.	mouse brain	10^{4}	(uncha-llenged)	33 90	>40 >40 >40 >40 >40 >40	
i.t.	diluent	0	(uncha-llenged)	10 33	<10 <10	

* Antibodies are quantified as the reciprocal of the dilution neutralizing cytopathic effect of 100 $TCID_{50}$ of the virus in 50% of the tubes
** Death after the challenge.

Intramuscular inoculation of primates

The 4 primates receiving the diploid cell-Tacaribe virus by i.m. route showed anti-Tacaribe antibody titers higher than 112 but no anti-Junin antibodies in the 2 tested monkeys. The monkeys showed absence of clinical signs, and resisted challenge with Junin virus in every case (Table 1).

Intrathalamic inoculation of primates

The 6 primates receiving Tacaribe virus by i.t. route showed no clinical signs; anti-Tacaribe serum antibodies were found when the animals were killed at 33 or 90 days p.i. (see Table 1). Histologic studies failed to show any pathologic changes, as may be seen in Fig. 1. No virus could be isolated from brain by suckling mouse inoculation.

Clinical or histologic alterations, virus in brain and antibodies were entirely lacking in the 2 primates that received diluent without virus. In 2 out of 8 marmosets inoculated by i.t. route, the needle's track could be found confirming the inoculation site.

Discussion

Nasal inoculation of *Callithrix jacchus* with Tacaribe virus induced serum neutralizing antibodies. All six primates that received the mouse brain-virus stock and 4 out of 6 that received the diploid cell-virus stock were protected against Junin virus challenge. The 2 primates which died showed titers of Junin virus in organs over a thousand-fold lower than those reported for primates receiving Junin virus alone (Weissenbacher *et al.*, 1982), suggesting that the drop in viral replication in primates with antibodies was apparently

not enough to prevent death. The efficacy of diploid cell Tacaribe virus for protection was demonstrated in 4 primates infected by i.m. route, which developed antibodies and protection against challenge with Junin virus. Heterologous anti-Junin antibodies could not be detected during the 82-day testing period, althrough their later appearance as known in the guinea pig (Weissenbacher *et al.*, 1975/6) cannot be excluded.

Previous reports showed that direct inoculation of virus in the CNS is a sensitive method for detection of neuropathogenicity, and that the nervous tissue seems to favour viral persistence (Johnson, 1982). Our data indicate that neither pathologic changes were observed in *Callithrix jacchus* inoculated i.t. with Tacaribe virus, nor viral persistence could be detected in brain, at least by the suckling mouse inoculation method. Infection of the primates was confirmed by the presence of anti-Tacaribe serum antibodies. Lack of neurovirulence in primates correlated with the lack of pathogenicity of Tacaribe virus previously observed in i.c. inoculated guinea pigs (Weissenbacher, unpublished). Accordingly, the model of i.t. inoculation of *Callithrix jacchus* might be a useful virulence marker. In addition, our findings suggest that the Tacaribe virus harmless, avirulent and protective against Junin virus in guinea pigs and in primates, may be a candidate for development of an experimental vaccine against AHF.

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