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Olive baboons: a non-human primate model for testing dengue virus type 2 replication

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SUMMARY

Objective: This study evaluated the use of a non-human primate, the olive baboon (*Papio anubis*), as a model of dengue infection. Olive baboons closely resemble humans genetically and physiologically and have been used extensively for assessing novel vaccine formulations.
Methods: Two doses of dengue virus type 2 (DENV-2) were tested in baboons: 10^3 and 10^4 pfu. Similarly, African green monkeys received the same quantity of virus and acted as positive controls.
Results: Following exposure, high levels of viremia were detected in both animal species. There was a trend to detect more days of viremia and more homogeneous viral titers in animals receiving the low viral dose. In addition, baboons infected with the virus generally exhibited positive virus isolation 1 day later than African green monkeys. Humoral responses consisting of antiviral and neutralizing antibodies were detected in all animals after infection.
Conclusions: We conclude that baboons provide an alternative non-human primate species for experimental DENV-2 infection and we recommend their use for further tests of vaccines, administering the lowest dose assayed: 10^3 pfu.

1. Introduction

Dengue is the most prevalent arthropod-borne viral disease in humans, with more than two billion people living in risk areas, and it is estimated that there are nine million symptomatic cases and 500 000 severe episodes of dengue worldwide each year.[1]

The dengue illness is transmitted by the bite of mosquitoes of the genus *Aedes*.[2] It is caused by four antigenically related but distinct dengue virus (DENV) serotypes (DENV-1 to DENV-4), which belong to the family *Flaviviridae*, genus *Flavivirus*.[2] Infection with DENV results in either asymptomatic or symptomatic disease, ranging from classical dengue fever (DF) to more severe cases of dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).[3] Despite the high incidence of this disease, there is currently no vaccine to prevent the spread of dengue disease or reduce its incidence worldwide. One of the issues that have hampered the development of the vaccine is the lack of a suitable animal model.[4]

Several species have been used to evaluate vaccine candidates in preclinical studies. Although non-human primates (NHP) do not develop the classical signs of dengue disease, this model is valuable for studying immune responses after infection with the virus and for evaluating several vaccine candidates.[5] After virus infection, susceptible animals develop viremia and humoral and cellular responses, but do not exhibit the classical clinical signs observed in humans.[5] The more common species employed have been rhesus (*Macaca mulatta*),[6–11] cynomolgus (*Macaca fascicularis*),[12] and New World monkeys.[13,14]

In previous studies, our group demonstrated that the African green monkey (*Chlorocebus aethiops sabaeus*) species is able to provide a potential model for preclinical assessment of novel vaccines.[15,16] In these animals, the mean viremia duration with DENV-2 was 1.6 to 5.6 days depending on the viral dose used.[15] On the other hand, when NHP were immunized with the vaccine candidate, this viremia period was reduced.[17]

This present study proposed to evaluate the olive baboon (*Papio anubis*) as a suitable animal model for DENV-2 infection. These NHPs belong to the group of Old World monkeys and are widely distributed in many parts of Africa, including northern, southern-central, and eastern Africa.[18] Their body size makes them

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particularly well-suited to a number of clinical procedures in preclinical studies,[19] and for the extraction of sufficient blood volumes during the period of viremia in order to test the parameters required for dengue vaccine assessment.

In the present study, two doses of DENV-2 were tested in baboons: 10^3 and 10^4 plaque-forming units (pfu). Similarly, African green monkeys received the same quantity of virus and acted as positive controls. It was possible to isolate the virus from all animals included in the study, and the animals developed a robust humoral immune response after virus infection. These results demonstrate the potential value of baboons as a model for dengue vaccine testing.

2. Materials and methods

2.1. Animals

Healthy young adult (2–5 kg) African green monkeys (*Chlorocebus aethiops sabaeus*) and healthy young adult (16–22 kg) olive baboons (*Papio anubis*), provided by the Institute of Primate Research (IPR, Nairobi, Kenya), were used in this study. All animals were screened for previous exposure to DENV by ELISA and plaque reduction neutralization test (PRNT). Animals were considered naive when antigen-specific antibodies were undetectable by ELISA (titer <1:50) or PRNT (titer <1:10). NHPs were maintained in accordance with the Kenyan guidelines for the care and use of laboratory animals, and all experimental procedures were approved by independent scientific and ethics committees at the IPR, Nairobi, Kenya.

2.2. Viruses

Infection was performed with a low-passage DENV-2 strain SB8553 (Asian genotype), isolated in 2002 from a human case of DF in Sibu, Malaysia (kindly provided by Dr M.J. Cardosa, University Sarawak, Malaysia). Aliquots of the infection virus were stored frozen at −80 °C at a titer of 2×10^6 pfu/ml. The standard strain New Guinea C of DENV-2 was used as sucrose–acetone antigen[20] for immunoassay tests.

2.3. Maintenance and clinical assessment

Animals were maintained throughout the study in individual cages that permitted conduct patterns to be evaluated according to the species, size, age, and sex. They were fed with commercial monkey chow, supplemented with fruits and vegetables. Water was available ad libitum. Infected baboons and African green monkeys were subjected to daily clinical inspections of lymphatic ganglia, skin, and respiratory, digestive, and nervous systems. The rectal temperature and body weight of all animals was assessed daily by the veterinary staff. Clinical biochemistry testing was performed on animals on day 0 and day 30 after infection, to avoid additional stress to the animals. These animals showed similar behavior to healthy animals; they did not show any signs of pain or distress at any time during the study. All results were reported and records maintained in accordance with IPR and Kenyan guidelines for animal use in research. The IPR follows international guidelines for the use of animals in biomedical research, as it is a World Health Organization Collaborating Center and has statutory registration with the NIH Office of Laboratory Animal Welfare, in addition to local and wide recognition in Africa as a Center of Excellence in preclinical studies.

2.4. Virus infection and detection of viremia

Six monkeys of each species were ranked by weight, age, and sex and then randomly divided into two groups of three animals each. Prior to each procedure, animals were anaesthetized with an intramuscular injection. African green monkeys were anaesthetized using ketamine hydrochloride, 10 mg/kg body weight. Baboons, due to their weight, were anaesthetized with a mixture of ketamine hydrochloride at 10 mg/kg and xylazine hydrochloride at 2 mg/kg body weight. Each animal was then infected subcutaneously with 1 ml (0.5 ml in each upper arm) of virus at doses of 10^3 or 10^4 pfu. The inoculums of 10^3 or 10^4 pfu were obtained by diluting a preparation of 2×10^6 pfu/ml in RPMI-1640 medium immediately prior to infection.

Starting on day 0, 4 ml of blood was collected daily from each animal for 11 days to detect viremia. For serological studies, the same volumes of blood were taken at day 30 post challenge. Animals were observed for 30 min after each inoculation for immediate local and systemic reactions and daily for the next 10 days.

Serum from clotted blood was stored at −70 °C until viremia was analyzed. The presence of virus in serum was determined by inoculating 140 μl of serum diluted 1:10 onto Vero cell monolayers grown in 25-cm^2 flasks. Fresh supplemented RPMI-1640 medium (5% heat inactivated fetal bovine serum, 2 mM L-glutamine, and 100 U of penicillin–streptomycin) was added. The cultures were incubated for 4 h at 37 °C in a 5% CO_2 atmosphere. Next, 2.5 ml of 3% medium-viscosity carboxymethylcellulose was added. Plates were incubated at 37 °C for 6 days in a 5% CO_2 atmosphere. To visualize the viral plaques, the monolayer was dyed with naphthol blue black solution (naphthol blue black 0.1%, sodium acetate 0.2 M, acetic acid 6%). Viremia was determined using the $\log_{10}$-transformed plaque counts detected on Vero cells ($\log_{10}$ pfu/ml).

2.5. Analysis of the antibody response

The anti-DENV IgG antibodies were monitored by ELISA. Briefly, flat-bottomed 96-well plates (Costar, USA) were coated with the monoclonal antibody 4G2, which recognizes the *Flavivirus* E protein,[21] in coating buffer (0.16% Na_2CO_3, 0.29% $NaHCO_3$, pH 9.5). Three washes with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (v:v) (PBS-T; Merck, Germany) were completed after each step. Plates were blocked with 2% bovine serum albumin (BSA), and then incubated overnight at 4 °C with a saturating concentration of DENV antigen and mock antigen in separate wells. Serially diluted samples from serum were incubated for 1 h at 37 °C with either the DENV or the mock antigen. Anti-monkey IgG-peroxidase conjugate (Sigma, USA) was added and the plates were incubated for 1 h at 37 °C. After washing, 0.04% substrate solution (*o*-phenylenediamine in buffer 2% Na_2HPO_4, 1% citric acid, and 30% H_2O_2, pH 5.0) was added. The plates were kept for 30 min at 25 °C and the reaction was stopped with 12.5% H_2SO_4. Absorbance was read at 492 nm in a microplate reader (SensIdent Scan; Merck, Germany). Titers were defined as the dilution of serum giving twice the absorbance value of the negative control serum.

Antibody functionality was measured by neutralization of DENV-2 (strain SB8553) infectivity by a PRNT on Vero cell culture as described previously.[22] The assay assessed four dilutions to calculate the 50% plaque end-point serum titers. The neutralizing antibody titer was identified as the highest serum dilution that reduced the number of virus plaques by 50% and was calculated by using a four-point linear regression method. The monoclonal antibody 4G2 was used as positive control, which recognizes the *Flavivirus* E protein.[21]

2.6. Statistical analysis

Data from the humoral antiviral immune response were assessed using the non-parametric Kruskal–Wallis test and Dunn