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Immune correlates of protection against yellow fever determined by passive immunization and challenge in the hamster model

Justin G. Julander[a], Dennis W. Trent[b,1], Thomas P. Monath[b,*]

[a] Institute for Antiviral Research, Utah State University, Logan, UT, USA
[b] Xcellerex Inc., 170 Locke Drive, Marlborough, MA 01752, USA

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ABSTRACT

Live, attenuated yellow fever (YF) 17D vaccine is highly efficacious but causes rare, serious adverse events resulting from active replication in the host and direct viral injury to vital organs. We recently reported development of a potentially safer β-propiolactone-inactivated whole virion YF vaccine (XRX-001), which was highly immunogenic in mice, hamsters, monkeys, and humans [10,11]. To characterize the protective efficacy of neutralizing antibodies stimulated by the inactivated vaccine, graded doses of serum from hamsters immunized with inactivated XRX-001 or live 17D vaccine were transferred to hamsters by the intraperitoneal (IP) route 24 h prior to virulent, viscerotropic YF virus challenge. Neutralizing antibody ($PRNT_{50}$) titers were determined in the sera of treated animals 4 h before challenge and 4 and 21 days after challenge. Neutralizing antibodies were shown to mediate protection. Animals having 50% plaque reduction neutralization test ($PRNT_{50}$) titers of ≥40 4 h before challenge were completely protected from disease as evidenced by viremia, liver enzyme elevation, and protection against illness (weight change) and death. Passive titers of 10–20 were partially protective. Immunization with the XRX-001 vaccine stimulated YF neutralizing antibodies that were equally effective (based on dose response) as antibodies stimulated by live 17D vaccine. The results will be useful in defining the level of seroprotection in clinical studies of new yellow fever vaccines.

1. Introduction

Yellow fever (YF), a mosquito-borne Flavivirus, causes hemorrhagic disease with jaundice in people inhabiting tropical areas of South America and Africa [1]. The incidence of YF was dramatically reduced following development of live, attenuated vaccines in the 1930s [1,2]. The live attenuated 17D vaccine, supplied by 7 manufacturers, is currently used to protect travelers and is incorporated in childhood vaccination programs in many countries, with millions of doses distributed annually.

While the live 17D vaccine is highly immunogenic and effective, it can cause serious adverse events associated with replication of the vaccine virus in vital organs [yellow fever vaccine associated viscerotropic disease (YEL-AVD) and neurotropic disease [(YEL-AND)]. Fortunately such events are rare [1,3], but the severity of these serious adverse events (case fatality rate 63% for YEL-AVD) is greater than any other vaccine. The risk of serious adverse events is higher in persons with certain underlying conditions that enhance replication of 17D virus, such as elderly and immunocompromised individuals [3,4].

The reporting rate of YEL-AVD and YEL-AND in the US is 0.4 and 0.8 per 100,000 vaccinations, respectively [4]. The highest rate of YEL-AVD (7.9 per 100,000) was observed during a recent immunization campaign in Peru [5]. The majority of cases of YEL-AVD have been reported since 2001 when these adverse events were first described [1,3], suggesting that earlier cases were frequently missed [6]. The reporting rate of serious adverse events is higher than for other vaccines including smallpox (0.29 per 100,000) [7] or oral polio (0.11 per 100,000) [8] that have been withdrawn for safety reasons. These considerations have stimulated recent efforts to develop safer YF vaccines [9].

XRX-001 is an inactivated, whole virion YF vaccine adjuvanted with aluminum hydroxide [10]. In studies of mice, hamsters and non-human primates, active immunization with XRX-001 elicited high titers of neutralizing antibodies (nAb) [10]. XRX-001 was also shown to elicit neutralizing antibodies in 100% of healthy human subjects, with geometric mean antibody titers >100 [11]. To confirm the critical role of nAb stimulated by the XRX-001 vaccine in protection against YF virus disease, and to identify the minimum protective nAb level required to prevent disease, passive antibody experiments were performed in a hamster model. Similar studies have previously been performed with various Flaviviruses, including YF [12–16]. These studies have demonstrated that pas-

* Corresponding author at: Kleiner Perkins Caufield & Byers, 2750 Sand Hill Rd., Menlo Park, CA 94025, USA. Tel.: +1 978 809 7045.
E-mail address: tmonath@kpcb.com (T.P. Monath).
[1] Present address: Office of Regulated Studies, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555, USA.

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sive transfer of nAbs provides protection against disease even when administered post-virus exposure, and that neutralizing activity is essential for protection.

The hamster-adapted Jimenez strain of YF virus causes viscerotropic disease in hamsters after IP inoculation [17]. This model has been used extensively in the evaluation of both antiviral drugs [18–21] and vaccines [9,16]. Outcome measures include serum levels of alanine aminotransferase (ALT), viremia and liver tissue virus, weight change, survival, and other serum chemistry parameters [22]. Using this model, we have determined the efficacy and minimum protective level of nAb stimulated by immunization with the inactivated XRX-001 and live 17D vaccines. The results of these studies will help to define the level of antibody that determines seroprotection in clinical trials.

2. Materials and methods

2.1. Animals

Female golden hamsters (*Mesocricetus auratus*) with an average weight of 100 g were sourced from Charles River Laboratories, Wilmington MA. After a 24-h quarantine and 7-day acclimation period, animals were randomly assigned to cages and individually marked with ear tags. All work with animals was performed in the Biosafety Level 3 (BSL-3) area of the AAALAC-accredited Laboratory Animal Research Center at Utah State University (USU). Hamsters were cared for in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council) under an animal use protocol approved by the IACUC at USU.

2.2. Viruses and virus infectivity assays

For use in neutralization tests, yellow fever 17D virus was prepared by performing a single passage of a commercial vial of YF-VAX® vaccine (sanofi pasteur, Swiftwater PA) in a monolayer culture of Vero cells (see source in Section 2.3) and by harvesting cell culture fluid at the appearance of cytopathic effects (CPE). The virus was quantified by plaque assay in Vero cells grown in 12 well plates under methylcellulose overlay, as previously described [10]. After 5 days of incubation at 37 °C and 5% CO_2, plates were fixed and stained with 0.3% crystal violet-formaldehyde and plaques were counted.

Hamsters were challenged with the Jimenez strain (South American genotype I, isolated in Panama, 1974). This virus had been adapted by serial passage in hamster liver, as described by Tesh et al. [17]. A seed stock was prepared from livers of hamsters removed 3 days after virus injection and homogenized in a 2× volume of sterile phosphate buffered saline. The liver homogenate had a titer of $10^{6.0}$ 50% cell culture infectious doses ($CCID_{50}$)/mL. Hamsters were challenged IP with 0.2 mL of a 10^{-4} dilution of virus stock, which is approximately 6.25 LD_{50}.

2.3. Vaccine

XRX-001 vaccine was manufactured in conformance to current Good Manufacturing Practices (cGMP) using a well-characterized Vero Cell Bank (WHO 10-87, passage 134, obtained from the American Type Culture Collection with permission of the US Food and Drug Administration) grown in serum-free medium. For vaccine production, Vero cells grown in serum-free medium on microcarrier beads were infected, the cell culture fluid was harvested when CPE appeared, and host cell DNA was digested with nuclease. The virus was purified by multiple filtration procedures, inactivated with β-propiolactone, further purified by cellufine sulfate chromatography, and adsorbed to 0.2% aluminum hydroxide. The potency of the final product is between 8.0 and 9.0 $\log_{10}$ virus equivalents (VE)/0.5 mL, VE being the inactivated antigen equivalent by ELISA of infectious virus determined by plaque assay [10,11].

2.4. Neutralization tests

Antibody levels in serum were quantified using the $PRNT_{50}$ as previously described [23]. Briefly, samples of test sera were heat-inactivated (56 °C, 30 min), serial twofold dilutions mixed with an equal volume of YF 17D virus containing 50–70 plaque forming units (PFU), incubated for 16–20 h at 2–8 °C, and inoculated onto wells of Vero monolayers grown in 12-well plates. After adsorption (1 h, 37 °C), monolayers were overlaid with 0.85% methylcellulose in Dulbecco's minimal essential medium (DMEM) containing 10% fetal calf serum. Plates were fixed and stained with crystal violet-formaldehyde after 5 days incubation at 37 °C. The endpoint was the highest dilution of serum inhibiting plaques by 50% or more compared to virus mixed with heat-inactivated fetal calf serum (negative control). XRX-001 immune serum served as a positive control.

2.5. Preparation of immune serum for use in passive immunization

Hamsters, 3–4 weeks of age were vaccinated by the intramuscular (IM) route on days 0 and 21 with XRX-001 at a dose of 8 $\log_{10}$ VE in 0.25 mL. Hamsters in the YF-VAX® vaccine control group received 0.1 mL (~4.0 $\log_{10}$ PFU) of undiluted YF-VAX® by the subcutaneous (SC) route and 10 hamsters in the placebo group received 0.2% alum in 0.9% NaCl by the IM route. Terminal blood collection was performed on day 49. Serum samples from individual hamsters within each group were pooled and the nAb titer of the pooled serum samples determined by $PRNT_{50}$. In one study (Experiment 2), serum from hamsters that received neither active nor placebo vaccinations was used as a control ('normal hamster serum').

2.6. Passive antibody transfer study design

For passive immunization studies, dilutions of pooled serum from the animals vaccinated with XRX-001, YF-VAX®, saline–alum placebo, or normal hamster serum were prepared as shown in Tables 1A and 1B. These dilutions were made based on estimates of the passive titer of nAbs in vivo, taking into consideration the dilution effects of hamster blood volume (assumed to be 7 mL), resulting in a 1:15 dilution of the 0.5 mL inoculum, and distribution in the intravascular space (assumed to be 50% of the IP inoculum), for an estimated total dilution in vivo of ~1:30. We were particularly interested in the protective capacity of very low passive antibody titers, because titers following active immunization <40 were previously shown to protect against challenge (10). A volume of 0.5 mL of each serum dilution was administered IP to groups of 10 hamsters 24 h prior to virus challenge. The $PRNT_{50}$ titers of the diluted serum pools used to passively immunize the hamsters are shown in Tables 1A and 1B, −24 h column, as well as the estimated passive titer following dilution in vivo.

Twenty-four hours after antibody treatment, animals were challenged with YF (Jimenez) virus and then followed for mortality, weight change, ALT levels, and viremia. Hamsters were challenged IP with 0.2 mL of a 10^{-4} dilution of virus stock, which is approximately 6.25 LD_{50}. Serum samples were collected from the retro-orbital sinus approximately 4 h before and 4 and 21 days after challenge to determine passive antibody titer by $PRNT_{50}$. ALT was determined on day 6 after challenge using a kit (Teco Diagnostics, Anaheim, CA) according to the manufacturer's directions and modified for use in 96-well plates. Viremia on day 4 post challenge was determined by testing four replicates of each of a series of