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Humanized monoclonal antibody 2C9-cIgG has enhanced efficacy for yellow fever prophylaxis and therapy in an immunocompetent animal model

Justin G. Julander [a], Brett A. Thibodeaux [b,c], John D. Morrey [a], John T. Roehrig [b], Carol D. Blair [c,*]

[a] Institute for Antiviral Research, Utah State University, Logan, UT 84322, USA
[b] Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO 80521, USA
[c] Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO 80523, USA

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ABSTRACT

Yellow fever virus (YFV) causes significant human disease and mortality in tropical regions of South and Central America and Africa, despite the availability of an effective vaccine. No specific therapy for YF is available. We previously showed that the humanized monoclonal antibody (MAb) 2C9-cIgG provided prophylactic and therapeutic protection from mortality in interferon receptor-deficient strain AG129 mice challenged with YF 17D-204 vaccine. In this study we tested the prophylactic and therapeutic efficacy of this MAb against virulent YFV infection in an immunocompetent hamster model. Intraperitoneal (ip) administration of a single dose of MAb 2C9-cIgG 24 h prior to YFV challenge resulted in significantly improved survival rates in animals treated with 380 or 38 μg of MAb compared to untreated animals. Treatment with the higher dose also resulted in significantly improved weight gain and reductions in serum alanine aminotransferase (ALT) and virus titers in serum and liver. Prophylactic treatment with 2C9-cIgG 24 h prior to virus challenge prevented the development of a virus-neutralizing antibody (vnAb) response in hamsters. Administration of a single ip dose of 380 μg of 2C9-cIgG as late as 72 h post-YFV challenge also resulted in significant improvement in survival rates. Hamsters treated at 4–72 h post-virus challenge developed a robust vnAb response. Enhanced survival and improvement of various disease parameters in the hamster model when MAb 2C9-cIgG is administered up to 3 days after virus challenge demonstrate the clinical potential of specific antibody therapy for YF.

1. Introduction

Infection with yellow fever virus (YFV) results in significant human morbidity and mortality in tropical regions of South and Central America and Africa. Like many other members of the *Flavivirus* genus, YFV is transmitted by mosquitoes. Although an effective vaccine is available for prevention of YF, it is estimated that 200,000 cases resulting in 30,000 deaths occur each year. Moreover, rare, severe vaccine-associated adverse events have been reported (Barrett and Teuwen, 2009), particularly in immunocompromised individuals. There is no approved treatment for cases of YF despite efforts to identify specific therapies (Julander, 2013).

Immune therapy, including passive administration of antibodies (Abs), is an effective method for treatment or prevention of infectious diseases (Keller and Stiehm, 2000). Limitations of this treatment are waning efficacy as the disease progresses, as well as difficulty in procuring sufficient quantities of human Ab for use in treatment. Humanization of mouse Ab combines the hypervariable regions of a murine monoclonal Ab (mMAb) gene specific to a given antigen with human Ab gene constant regions, and permits ease of production and use of clinically safe MAbs for the treatment of various human diseases.

The mMAb 2C9, which reacts with amino acids 71, 72, and 125 in domain II of the YFV envelope protein, has been shown previously to prophylactically protect 6-week-old mice from disease following intracerebral inoculation with YFV (Brandriss et al., 1986; Lobigs et al., 1987). We used MAb 2C9 to develop a human-mouse chimeric MAb, 2C9-cIgG, for evaluation of prophylactic and therapeutic efficacy for YFV infections.

Various animal models have been useful in the evaluation of investigational antiviral therapies for YF, including Ab treatment.

* Corresponding author. Address: Department of Microbiology, Immunology and Pathology, 1692 Campus Delivery, Colorado State University, Fort Collins, CO 80523-1692, USA. Tel.: +1 970 491 8243; fax: +1 970 491 8323.
E-mail addresses: cblair@colostate.edu, carol.blair@colostate.edu (C.D. Blair).

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Rhesus monkeys develop YF disease that is very similar to that observed in humans, although the course of disease tends to be more rapid, with death occurring within one week after infection (Monath et al., 1981). Rhesus monkeys were demonstrated to be useful models for immune therapy; they were protected from lethal YFV challenge by Ab administration up to 3 days post-infection (dpi), although immune serum treatment had no therapeutic effect if initiated after the onset of disease (Monath, 2008).

Mice typically develop encephalitis when infected with YFV, making them less well-suited as models of human disease. We recently developed a murine model of disease in mouse strain AG129, which is deficient in α/β- and γ-interferon receptors, peripherally challenged with YF 17D-204 vaccine (Thibodeaux et al., 2012a) and used this model system for evaluation of 2C9-cIgG (Thibodeaux et al., 2012b). Although AG129 mice lack a functional interferon response, they provide a useful model for initial proof of concept studies. We showed that both murine 2C9 and 2C9-cIgG protected AG129 mice from peripheral challenge with YFV 17D-204 when administered prophylactically 24 h prior to infection at antibody concentrations $\geqslant$1.27 μg/mouse and exhibited therapeutic activity when administered at 127 μg/mouse up to 24 h post-infection (hpi).

Infection of hamsters with the hamster-adapted Jimenez strain of YFV results in viscerotropic disease that is similar in many ways to human disease (Julander et al., 2007; Sbrana et al., 2006; Tesh et al., 2001; Xiao et al., 2001). Virus titer in serum peaks 4 days after virus challenge with mortality observed as early as 5 or 6 dpi. Relevant disease parameters, including increases in serum alanine aminotransferase (ALT), weight loss, and morbidity can be measured for evaluation of experimental antiviral therapeutic efficacy. This model has been used to evaluate a number of antiviral treatments, including passively administered neutralizing Abs (Julander et al., 2011). This hamster model was used in the present study to determine the efficacy of 2C9-cIgG in an immunocompetent and relevant model of YF.

2. Materials and methods

2.1. Animals

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The study was conducted in accordance with protocol number 1231 approved by the Institutional Animal Care and Use Committee of Utah State University. Experiments were conducted in the AAALAC-accredited BSL-3 animal suite at the Utah State University Laboratory Animal Research Center (LARC). All LARC personnel continually receive special training on blood-borne pathogen handling by the university's Environmental Health and Safety Office. Female Syrian golden hamsters with an average weight of 99 g were used after a quarantine period greater than 48 h. Animals were randomly assigned to cages and individually marked with ear tags. Animals were observed at least twice daily for signs of morbidity and euthanized when severe morbidity occurred. Every effort was made to minimize suffering.

2.2. Antibody and virus

A stock of purified 2C9-cIgG was commercially prepared (QED Bioscience, San Diego, CA). The MAb was diluted in phosphate-buffered saline (PBS). A hamster dose was determined using results from the AG129 mouse studies (Thibodeaux et al., 2012b), based on surface area conversion. The West Nile virus (WNV)-specific, humanized MAb MGAWN1 was obtained from Macrogenics Inc. (Rockville, MD). Reagents were prepared in sterile saline immediately prior to initial administration and stored at 4 °C.

The hamster-adapted (p. 10) Jimenez strain of YFV was a generous gift from Robert B. Tesh (University of Texas Medical Branch, Galveston, TX). To prepare viral stocks, five adult female hamsters were infected by intraperitoneal (ip) inoculation of virus as described for the original hamster adaptation (Tesh et al., 2001). The livers of the infected hamsters were removed 3 dpi and homogenized in a 2× volume of sterile PBS. This liver homogenate supernatant had a titer of $10^{6.0}$ 50% cell culture infectious doses ($CCID_{50}$)/ml.

2.3. Animal challenge experiments

Although dose and timing of 2C9-cIgG administration differed between studies, all antiviral experiments had the same basic design. Hamsters were randomly assigned to groups of 5–21 animals. A single ip administration of 2C9-cIgG was used in all antiviral studies. The WNV-specific, humanized MAb hE16 (MGAWN1) (Oliphant et al., 2005) was included as a nonspecific MAb control in all studies at concentrations matching the highest dose of 2C9-cIgG. Ribavirin (provided by ICN Pharmaceuticals, Inc., Costa Mesa, CA), administered ip twice daily for 6 days beginning 4 h before infection at a dose of 50 mg/kg/d, was included in prophylaxis studies as a positive treatment control. Hamsters were challenged ip with 0.1 ml of virus administered bilaterally (0.2 ml total) at a virus concentration of $10^{2.0}$ $CCID_{50}$/ml (20 $CCID_{50}$/animal). Serum was collected 4 dpi to measure virus titer and from all surviving hamsters 6 dpi for quantitation of ALT. For quantitation of virus-neutralizing Ab (vnAb), serum was collected 4 h prior to virus challenge and at the termination of the study on 21 dpi. Hamsters were observed at least twice daily for morbidity, and weights were determined 0, 3, 5 and 6 dpi. Initial signs of morbidity occurred 4 dpi and moribund animals that were immobile were humanely euthanized. A group of mock-infected animals treated with 2C9-cIgG were included in the initial study as a toxicity control and uninfected, untreated normal controls were included in each experiment.

2.4. Infectious virus assays

Hamster liver homogenate supernatants and serum samples were serially diluted from 10^{-1} to 10^{-8} and 0.1 ml of each dilution was added to each of four wells of a 96-well plate containing confluent Vero 76 cells. Ten days later cytopathic effects (CPE) in each well were used to identify the end-point of infection for calculation of the $CCID_{50}$/ml. Results were expressed as $CCID_{50}$ per ml serum or g tissue for comparison.

2.5. Virus-neutralizing antibody assays

For quantitation of vnAb, a standard 50% plaque-reduction neutralization test ($PRNT_{50}$) was performed on Vero cells using previously published protocols (Julander et al., 2011).

2.6. Serum alanine aminotransferase assays

Serum was collected *via* bleeding from the ocular sinus 6 dpi. Alanine aminotransferase (ALT) SGPT reagent (Teco Diagnostics, Anaheim, CA) was used in the enzymatic assay, and the protocol was altered for use in 96-well plates as described previously (Julander et al., 2007). The ALT concentrations were determined per manufacturer's instructions.