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Human polyclonal antibodies produced in transchromosomal cattle prevent lethal Zika virus infection and testicular atrophy in mice

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ABSTRACT

Zika virus (ZIKV) is rapidly spreading throughout the Americas and is associated with significant fetal complications, most notably microcephaly. Treatment with polyclonal antibodies for pregnant women at risk of ZIKV-related complications could be a safe alternative to vaccination. We found that large quantities of human polyclonal antibodies could be rapidly produced in transchromosomal bovines (TcB) and successfully used to protect mice from lethal infection. Additionally, antibody treatment eliminated ZIKV induced tissue damage in immunologically privileged sites such as the brain and testes and protected against testicular atrophy. These data indicate that rapid development and deployment of human polyclonal antibodies could be a viable countermeasure against ZIKV.

1. Introduction

In response to the global spread of Zika virus (ZIKV), there has been a rapid scale-up in the development of vaccines and therapeutics. ZIKV has been causally linked to microcephaly, congenital malformation, intrauterine growth retardation, and Guillain-Barre syndrome (Brasil et al., 2016; Cao-Lormeau et al., 2016; Cao-Lormeau and Musso, 2014; Mlakar et al., 2016). Several reports have been published identifying a DNA vaccine expressing the pre-membrane (preM) and envelope protein (E) protein of ZIKV that provides significant protection from infection in mice (Muthumani et al., 2016), as well as non-human primates (NHP's) (Abbink et al., 2016; Dowd et al., 2016; Larocca et al., 2016). Sexual transmission and persistence in the semen after systemic clearance of the virus has been extensively reported since the initial stages of the outbreak (D'Ortenzio et al., 2016; Emanuele Nicastri, 2016). The widespread use of the Ifnar1$^{-/-}$ mouse model has led to the discovery of testicular atrophy in mice surviving ZIKV challenge, with ongoing replication in immune privileged sites (Govero et al., 2016; Ma et al., 2016; Uraki et al., 2017). This highlights the importance of controlling replication in sites such as the brain (especially in developing fetuses) as well as the reproductive tract. Another option for treatment of infection, particularly in pregnant women, could be the use of therapeutic antibodies. Antibody-based therapies, such as RhoGAM® are currently used in Rh-negative pregnant women to prevent immune responses against a Rh-positive fetus (Pollack et al., 1968). More recently monoclonal antibody approaches have been used to prevent emerging infectious diseases, most notably the outbreak of Ebola virus (EBOV) in West Africa. Indeed, a cocktail of monoclonal antibodies called ZMapp™, in

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some cases was found to be an effective post-exposure treatment for EBOV disease in humans. However the rapid scale up of the ZMapp™ cocktail in an outbreak setting proved to be difficult (PREVAIL II Writing Group et al., 2016). It is also becoming increasingly clear that in order to provide sufficient protection and prevent the emergence of antibody-escape mutants, cocktails of monoclonal antibodies targeting various viral epitopes are required. Despite some of these challenges, several recent studies have shown considerable efficacy of antibody-based therapies in animal models (Abbink et al., 2016; Sapparapu et al., 2016; Wang et al., 2016).

Recently, genetically-engineered cattle were produced, in which both of the bovine immunoglobulin (Ig) heavy chain loci (*IGHM* and *IGHML1*) and lambda light chain cluster genes (*IGL*) were genetically inactivated (triple knockout; *IGHM*$^{-/-}$ *IGHML1*$^{-/-}$ *IGL*$^{-/-}$) and the Ig functions were reconstituted by an artificial chromosome comprising the entire human Ig heavy chain and kappa chain repertoire in their germline configurations (Kuroiwa et al., 2009; Matsushita et al., 2014, 2015; Sano et al., 2013). The resultant transchromosomal bovines (TcB) produce target-specific, fully human polyclonal IgG antibodies following hyperimmunization. This platform greatly reduces the scale-up time of large quantities of purified human IgG, compared to traditional hybridoma antibody platforms. By merging the TcB system with gene-based vaccine technology, it is possible to focus the antibody responses against the most antigenic portions of a particular infectious agent (e.g. virus envelope glycoproteins). The use of gene-based vaccine strategies negates the need for isolation or antigen production from the infectious agent for antibody production. Recently, as a proof of concept for the treatment of hantavirus pulmonary syndrome, Hooper et al. demonstrated that fully human antibody produced in TcB, using a combination of two DNA vaccines, could protect Syrian hamsters from lethal disease when administered up to 5 days post-infection (dpi) (Hooper et al., 2014). A similar study demonstrated that potent and protective neutralizing antibody could be produced in TcB vaccinated with a combination of EBOV DNA vaccines (Bounds et al., 2015). Thus the TcB system is a promising solution to rapidly evolving outbreak situations.

In this study, TcB were vaccinated using a DNA vaccine expressing the preM/E protein of Zika virus. Fully human polyclonal antibodies were subsequently produced against the Zika virus glycoprotein with high neutralizing titers. These antibodies were then assessed for their ability to protect against a lethal challenge of Zika virus in two mouse models. Concurrent as well as delayed treatment with respect to challenge showed significant protection against lethal disease. Additionally, treatment eliminated virus induced tissue damage and protected against testicular atrophy. These data indicate that rapid development and deployment of human polyclonal antibodies produced in TcB could be a viable countermeasure against rapidly emerging infectious diseases like Zika virus.

2. Materials & methods

2.1. Ethics

The Public Health Agency of Canada Animal Care Committee under the Animal Use Document, H-16-009, approved all Ifnar1$^{-/-}$ in-vivo experiments for this study. All procedures were conducted by certified personnel, adhering to the guidelines set out by the Canadian Council on Animal Care. Studies using wildtype C57BL/6 mice were approved by a standing internal institutional animal care and use committee (IACUC) and were conducted in compliance with the United States Animal Welfare Act and other federal statutes and regulations relating to animals. Additionally all experiments involving animals adhere to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council. Both Ifnar1$^{-/-}$ and C57BL/6 animals meeting the predetermined scoring criteria were humanly euthanized.

2.2. Viruses

ZIKV strain DAK ARD 41525 isolated in 1984 from *Aedes africanus* mosquitoes in Senegal was obtained from the World Reference Center for Emerging Viruses and Arboviruses (R. Tesh, University of Texas Medical Branch). This virus was amplified once in AP61 and C6/36 cells, and three times in Vero cells prior to use (Ladner et al., 2016). A Puerto Rican ZIKV isolate was also used for the Ifnar1$^{-/-}$ mouse challenges (*Homo sapiens*/PRI/PRVABC59/2015, GenBank accession no. KX087101.2).

2.3. Mice

Wild-type C57BL/6 mice and homozygous Ifnar1$^{-/-}$ knockout mice (B6.129S2-Ifnar1^{tm1Agt}/Mmjax) were obtained from The Jackson Laboratory (Bar Harbor, USA). Ifnar1$^{-/-}$ mice have a genetic disruption in the IFN-I receptor 1 domain and accordingly do not respond to known type I IFNs. Inoculations were performed under isoflurane gas anesthesia, and all attempts were made to minimize animal suffering related to the procedures and disease progression throughout the study. Animals were monitored daily for 28 days for survival, weight, and clinical signs of disease.

2.4. Transchromosomal bovines (TcB)

TcB were produced as previously described (Kuroiwa et al., 2009; Matsushita et al., 2015, 2014; Sano et al., 2013). Briefly, the TcB used in this study is homozygous for triple knock-outs in the endogenous bovine immunoglobulin genes (*IGHM*$^{-/-}$ *IGHML1*$^{-/-}$ *IGL*$^{-/-}$) and carry a human artificial chromosome (HAC) vector labeled as KcHACD. This HAC vector consists of two human chromosome fragments: the A14 fragment contains the entire human immunoglobulin heavy chain locus except that the IGHM constant region remains bovine and the key regulatory sequences were bovinized; and a human chromosome 2 fragment contains the entire human immunoglobulin κ light chain locus.

2.5. Vaccine construction

The codon-optimized full-length ZIKV prM/E genes were *de novo* synthesized (GeneWiz; South Plainfield, NJ) in frame with a 5′ synthetic Japanese encephalitis (JE) leader sequence MGKRSAGSIMWLASLAVVIACAGA (Davis et al., 2001). The prM/E sequence was derived from NCBI sequence for a Brazilian strain of ZIKV (KU312314.1). Genes were cloned into the *Not*I and *Bgl*II sites of the pWRG7077 vector and verified by sequence analysis to produce the pWRG/ZIKV-JE-prME(opt). Expression of the ZIKV glycoproteins was confirmed in cell culture by immunofluorescence using a cross-reactive anti-Dengue monoclonal antibody (MAb-4G2). The plasmid was shown to elicit neutralizing antibodies in a rabbit after vaccination with the PharmaJet i.m. DSJI device leading to a $PRNT_{80}$ geometric mean titer of 3225 after four vaccinations (Fig. S1).

2.6. Immunization of transchromosomal bovines

One TcB (#2227) was immunized with ZIKV pDNA vaccine at 12 mg per animal per vaccination by using the PharmaJet Stratis® IM injection device as previously described (Sano et al., 2013). Each vaccine was administered with two injections of 3 mg each on each side of hind leg (for 6 mg total per site). One ml of SAB-adj-1 was