Virology ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Vaccination with recombinant adenoviruses expressing Ebola virus glycoprotein elicits protection in the interferon alpha/beta receptor knock-out mouse

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ARTICLE INFO

Article history:
Received 11 February 2013
Returned to author for revisions
6 March 2013
Accepted 27 March 2013

Keywords:
Ebola virus
Interferon α/β receptor knock-out mouse
Small animal model
Recombinant adenovirus vaccine

ABSTRACT

The resistance of adult immunocompetent mice to infection with ebolaviruses has led to the development of alternative small animal models that utilise immunodeficient mice, for example the interferon α/β receptor knock-out mouse (IFNR$^{-/-}$). IFNR$^{-/-}$ mice have been shown to be susceptible to infection with ebolaviruses by multiple routes but it is not known if this murine model is suitable for testing therapeutics that rely on the generation of an immune response for efficacy. We have tested recombinant adenovirus vectors for their ability to protect IFNR$^{-/-}$ mice from challenge with Ebola virus and have analysed the humoral response generated after immunisation. The recombinant vaccines elicited good levels of protection in the knock-out mouse and the antibody response in IFNR$^{-/-}$ mice was similar to that observed in vaccinated wild-type mice. These results indicate that the IFNR$^{-/-}$ mouse is a relevant small animal model for studying ebolavirus-specific therapeutics.

Introduction

The *Ebolavirus* genus is contained within the *Filoviridae* family and consists of enveloped viruses with non-segmented, single-strand, negative-sense RNA genomes (Sanchez et al., 2007). Serological and genetic analysis has identified five distinct species of ebolavirus: *Zaire ebolavirus*, *Sudan ebolavirus*, *Taï Forest ebolavirus*, *Reston ebolavirus* and *Bundibugyo ebolavirus* (Falzarano et al., 2011; Towner et al., 2008). Ebolaviruses can cause outbreaks of severe haemorrhagic fever in humans and non-human primates with up to 90% fatality rates reported in humans (Feldmann et al., 2003). Transmission of ebolaviruses is generally due to direct contact with blood, secretions or infected tissues although there is also evidence for an airborne route of infection (Jaax et al., 1995; Johnson et al., 1995; Roels et al., 1999).

Licensed vaccines and antivirals are currently not available for the treatment of ebolaviruses but there is an urgent requirement for their development due to continual sporadic outbreaks and the potential for use in a bioterrorist attack (Borio et al., 2002). Although non-human primates are believed to be the animal model most representative of human disease (Bente et al., 2009; Bray and Paragas, 2002), ethical, practical and financial considerations have meant that initial efficacy screening has commonly been performed in small animal models. Unlike non-human primates, guinea pigs and adult immunocompetent mice are not susceptible to infection with naturally occurring ebolaviruses and this has led to the development of host-adapted viruses which are able to cause lethal disease in these small animals. Thus, therapeutics for ebolaviruses are often initially tested against adapted virus in a mouse or guinea pig model. They may then progress to testing in non-human primates (Rhesus and Cynomolgus macaques or African green monkeys) if efficacy is observed (Bente et al., 2009). However, positive results obtained with adapted viruses in small animal models have generally not translated into successful outcomes against ebolaviruses in the non-human primate model (Falzarano et al., 2011; Geisbert et al., 2002).

The current and most extensively used murine model employs a mouse-adapted strain of Ebola virus (Bray et al., 1998). Adaptation to mice through sequential passage resulted in a number of nucleotide changes in both coding and non-coding regions of the viral genome (Ebihara et al., 2006). The mutations principally affected the ability of Ebola virus to overcome the type I interferon (IFN) response but other mutations also contributed to the virulent phenotype. Consequently, mouse-adapted Ebola virus does not reflect natural viral properties. Whilst there are some biochemical and pathological similarities between mice infected with the adapted virus and those observed in non-human primates infected with wild-type Ebola virus (EBOV), certain differences are observed,

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0042-6822/$ - see front matter © 2014 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.virol.2013.03.028

for example in levels of fibrin deposition (Bray et al., 2001). Additionally, the adapted virus is only lethal to mice when administered by the intraperitoneal route (Bente et al., 2009) whereas non-human primates are susceptible to infection by multiple routes. This small animal model therefore has considerable limitations.

Although the precise mechanisms of ebolavirus disease are not known, the inhibition of type I IFN responses is believed to be crucial. Indeed, ebolaviruses encode two viral proteins, VP24 and VP35, that block IFN responses (Ramanan et al., 2011). Treatment of non-human primates with IFN-α2b prolonged survival and delayed the development of viraemia (Jahrling et al., 1999) and, in humans, it has been shown that fatal infections with EBOV are associated with a lack of an IFN α2 response (Wauquier et al., 2010). The resistance of adult immunocompetent mice to ebolavirus infection is believed to be a consequence of the robust innate immune response, particularly the type I IFN response, of these animals (Bray, 2001; Ebihara et al., 2006). A more suitable small animal model of human infection would initiate a less vigorous innate immune response upon administration of ebolavirus. Accordingly, an alternative murine model of ebolavirus infection utilises the IFN α/β receptor knock-out (IFNR$^{-/-}$) mouse (Müller et al., 1994). EBOV is able to establish a lethal infection in this model by either the intraperitoneal (Bente et al., 2009; Lever et al., 2012), subcutaneous (Bente et al., 2009) or aerosol route (Lever et al., 2012). The pathology of infection in the IFNR$^{-/-}$ mouse is similar to that observed in non-human primates infected with ebolaviruses by various routes (Lever et al., 2012) and encompasses characteristic pathological features observed in humans (Lever et al., 2012). In addition to susceptibility to infection with EBOV, this mouse strain has been shown to be susceptible to Sudan virus (Bray, 2001) as well as Marburg virus (Bray, 2001; Lever et al., 2012).

As a first step in determining whether the IFNR$^{-/-}$ mouse could be a more appropriate small animal model for ebolavirus infection than the use of adapted virus in adult immunocompetent mice, we investigated the ability of this knock-out mouse strain to respond to adenovirus vectors expressing full-length and truncated forms of EBOV glycoprotein (GP). The GP gene of ebolaviruses has three overlapping open-reading frames which encode three proteins: GP, secreted glycoprotein (sGP) and secondary secreted glycoprotein (ssGP). The transmembrane surface GP, which mediates receptor binding and membrane fusion, is only produced after transcriptional RNA editing of the GP gene and proteolytic processing (Sanchez et al., 1996; Volchkov et al., 1998). The principal product of the GP gene is the non-structural sGP which is expressed from non-edited mRNA (Volchkova et al., 1998). sGP is secreted from infected cells and shares the N-terminal 295 amino acids with GP but differs in the C-terminal by 69 amino acids (Volchkova et al., 1998). Another editing product of the GP gene, ssGP, is also secreted from cells and shares the 295 N-terminal amino acids with sGP but lacks the C-terminal amino acids (Volchkova et al., 1998). During virus replication in vivo, 67% of GP gene-specific mRNAs direct synthesis of sGP, 31% direct expression of GP and 2% direct expression of ssGP (Mehedi et al., 2011). We constructed recombinant human adenovirus type 5 (RAd) expressing GP, sGP or ssGP and tested the ability of each vaccine to protect IFNR$^{-/-}$ mice from EBOV infection. Adenovirus-vectored GP is known to protect non-human primates from ebolavirus infection (Pratt et al., 2010; Sullivan et al., 2006; Swenson et al., 2008) and was chosen for this work as an established efficacious vaccine. However, the protective efficacy of sGP or ssGP has not been investigated. The humoral response of vaccinated IFNR$^{-/-}$ mice was also compared to that generated in mice of the wild-type (WT) parental strain to determine how the absence of an IFN α/β receptor affected the antibody response to the three recombinant vaccines.

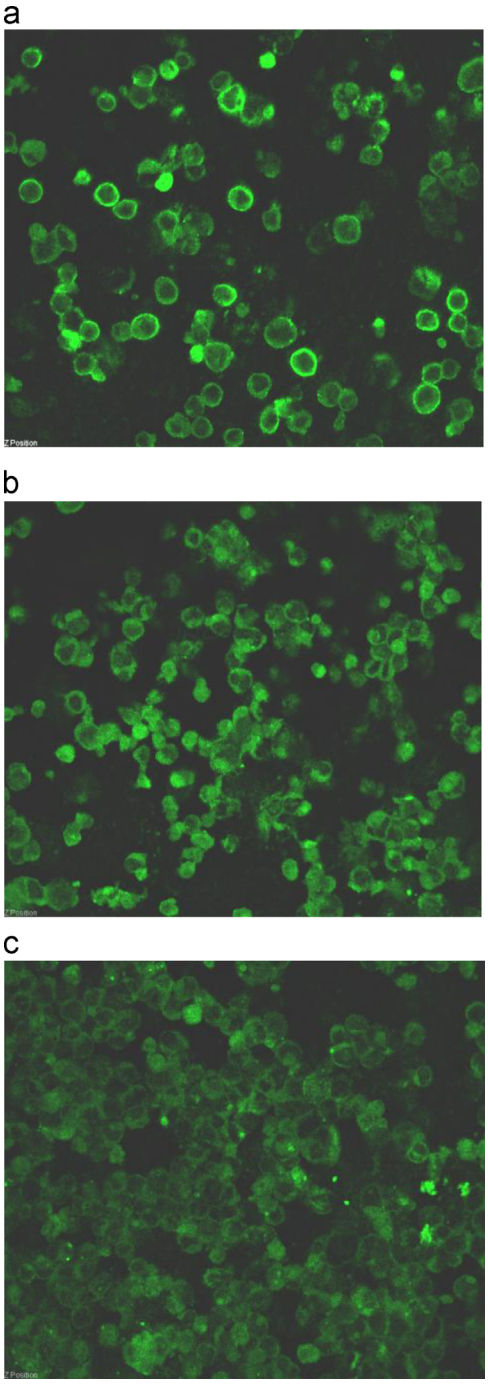

Fig. 1. Detection of EBOV antigen expression by immunofluorescence. HEK 293 cells infected with RAd/GP (a), RAd/sGP (b) or RAd/ssGP (c) were fixed in cold acetone and stained with polyclonal anti-EBOV followed by anti-rabbit IgG (whole molecule) conjugated to FITC. Images of representative fields of view under UV illumination were captured using a confocal microscope.

Results

RAd/GP, RAd/sGP and RAd/ssGP express the full-length and truncated variants of GP

The expression of viral glycoprotein antigen was confirmed by staining cells infected with either RAd/GP, RAd/sGP or RAd/ssGP with rabbit polyclonal anti-EBOV immunoglobulin (Fig. 1). Fluorescence was not observed with cells infected with the empty adenovirus vector, RAd (results not shown).

Please cite this article as: O'Brien, L.M., et al., Vaccination with recombinant adenoviruses expressing Ebola virus glycoprotein elicits protection in the interferon alpha/beta receptor knock-out mouse. Virology (2014), http://dx.doi.org/10.1016/j.virol.2013.03.028