Short Communication

Intranasal immunization with an adenovirus vaccine protects guinea pigs from Ebola virus transmission by infected animals

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ABSTRACT

Experimental Ebola virus (EBOV) vaccines have previously been shown to protect animals against a high dose intramuscular (IM) challenge, which is seen as a stringent challenge model. However, the protective efficacy against other modes of infection, such as contact with infectious hosts, is unknown. Using a previously established EBOV transmission animal model, we evaluated the efficacy of an adenovirus-based EBOV vaccine given to guinea pigs (gps) 4 weeks before direct contact with untreated, infectious animals. Prior vaccination resulted in robust levels of EBOV-specific antibodies and conferred complete protection in gps. These results support the use of vaccines to prevent EBOV transmission between hosts.

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1. Introduction

EBOV is one of the most virulent agents in humans and has caused sporadic outbreaks primarily localized to the humid, remote rainforests of sub-Saharan Africa since its discovery in 1976. Initial symptoms of EBOV infection are similar to that of other more common pathogens, such as fever, nausea, diarrhea and general malaise before a rapid progression to more specific indications including hemorrhage, multi-organ failure and a condition resembling septic shock (Feldmann and Geisbert, 2011). Death is the outcome in up to 90% of human cases, occurring between 6 and 10 days after the onset of symptoms (Feldmann and Geisbert, 2011). Clinically approved prophylaxis and post-exposure therapeutics currently do not exist for victims of EBOV infection. Due to the size and scale of the 2014 EBOV outbreak in West Africa, several experimental vaccines are being fast-tracked through clinical trials in an attempt to protect frontline health workers and limit the number of new infections. The vaccine candidates currently being fast-tracked include those based on the vesicular stomatitis virus (Jones et al., 2005) or the chimpanzee-derived adenovirus

serotype 3 platforms (Stanley et al., 2014). Other potential vaccine candidates slated for upcoming clinical trials include the human adenovirus serotype 26 or 35 platforms (Geisbert et al., 2011) with a Modified vaccinia Ankara (MVA) boost. However, the EBOV challenge for these vaccine studies in nonhuman primates (NHPs) is typically an IM infection with a target dose of 1000 plaque forming units (PFU), which is designed to test whether immunization in advance is able to protect recipients under stringent challenge conditions, such as an accidental needlestick injury in the laboratory. While important, this scenario may not be relevant to outbreak situations, in which transmission between humans likely occurs through direct contact with infected materials, such as infected bodily fluids and tissues (Cohen, 2004) in a nosocomial setting or during traditional burial rituals. As a result, there is an urgent need to test whether experimental EBOV vaccines are efficacious against a more common infection route.

A transmission model was recently developed in mucosally-infected gps, which is one possible route of EBOV infection in animals (Twenhafel et al., 2015). In this model, an intranasal (IN) challenge with gp-adapted EBOV (GA-EBOV) resulted in earlier virus shedding by the infected animal, leading to increased virus transmissibility to naïve gps added as cagemates 24 h after infection (Wong et al., 2014). In addition, a human adenovirus serotype 5 (Ad5)-based vaccine and adjuvant combination (hereafter termed adjuvanted Ad5-ZGP) was previously shown to confer complete protection in gps challenged systemically by GA-EBOV at high

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doses (Richardson et al., 2011). Using adjuvanted Ad5-ZGP as a candidate vaccine, our aim is to determine whether immunization of contact gps 4 weeks before direct exposure to an untreated, infectious animal is effective at protecting the vaccine recipient from EBOV transmission. Survival and clinical symptoms, in addition to viremia and EBOV shedding of contact animals were monitored to confirm vaccine efficacy. Since the specific antibody response statistically correlates with survival from EBOV (Wong et al., 2012), total IgG and neutralizing antibodies were quantified before and after challenge from contact animals as an additional indicator of vaccine-induced protection.

To investigate whether vaccination is able to prevent the susceptibility of animals to contact EBOV transmission, a group of 3 naïve contact gps were administered IN with 1.2×10^9 infectious forming units (IFU) of Ad5-lacZ, and groups of 6 naïve contact gps were given IN with PBS or 1.0×10^9 IFU Ad5-ZGP vaccine combined with 2.0×10^8 IFU Ad5-IFNα adjuvant. Four weeks after vaccination, the immunized animals were individually paired-housed with unvaccinated animals ($n = 15$) that had been challenged 24 h previously via the IN route with $10,000 \times LD_{50}$ (220 PFU) of GA-EBOV (Ebola virus VECTOR/C. porcellus-lab/COD/1976/Mayinga-GPA, order Mononegavirales, family Filoviridae, species Zaire ebolavirus; Genbank accession number AF272001.1) (Connolly et al., 1999). The animals were then monitored over the next 4 weeks for survival and changes in weight or body temperature. All challenged gps succumbed to disease between 8 and 11 dpi, with a mean time to death of 8.9 ± 1.0 dpi. From contact gps, PBS-treated control animals resulted in 17% survival, with 5 of 6 animals succumbing to EBOV between 13 and 16 dpi (Fig. 1A). In contrast, 100% survival was observed in gps given adjuvanted Ad5-ZGP, with 0 of 6 animals succumbing to disease (Fig. 1A). Significant weight loss or abnormal body temperatures were not observed in any animals from this vaccination group (Fig. 1B and C). Ad5-lacZ administration yielded 33% survival, with 2 of 3 gps succumbing to EBOV at 17 and 21 dpi (Fig. 1A). Using the Logrank Mantel–Cox test, the overall survival between the individual contact groups was shown to be statistically significant ($\chi^2 = 9.555$, df = 2, p-value = 0.0084).

Levels of GA-EBOV shedding from contact gps of various immunization groups were measured by blood, oral, nasal and rectal swabs sampled at 14 and 28 dpi using a previously described protocol (Qiu et al., 2013). Samples were harvested at 14 dpi in order to compare GA-EBOV viremia and shedding between the various groups of vaccinated contact gps, and at 28 dpi in order to determine whether vaccinated survivors still harboured any virus. GA-EBOV RNA could be detected from oral, nasal and rectal swabs in only moribund animals given PBS at $\sim10^5$, $\sim10^4$, and $\sim10^5$ GEQ/mL respectively at 14 dpi, as well as oral secretions of animals given Ad5-lacZ at $\sim10^3$ GEQ/mL, at 14 dpi. In addition, viremia was observed in both the PBS and Ad5-lacZ groups, between $\sim10^5$ and $\sim10^7$ GEQ/mL at 14 dpi (Fig. 1D). In contrast, GA-EBOV RNA was not detected in the blood, as well as oral, nasal and rectal secretions of gps vaccinated IN with adjuvanted Ad5-ZGP at 14 dpi (Fig. 1D). GA-EBOV RNA was also not detected in surviving animals upon termination of the experiment at 28 dpi (data not shown).

To determine whether vaccination resulted in specific humoral immune responses, sera were harvested from all contact animals before vaccination (26 days before infection of challenge animals), on the day of challenge (0 dpi), as well as 14 and 28 dpi. IgG antibodies and neutralizing antibodies specific for the EBOV glycoprotein (ZGP) or virion were measured by ELISA and neutralizing antibody assays, respectively, using a previously established protocol (Qiu et al., 2013). The administration of PBS or Ad5-lacZ did not yield significant levels of specific antibody responses against GA-EBOV (Fig. 2A and B). Significant levels ($p < 0.0001$) of ZGP-specific IgG were observed for gps given adjuvanted Ad5-ZGP, with endpoint titers of $110,000 \pm 33,000$, $171,000 \pm 66,100$ and $192,000 \pm 70,100$ at 0, 14 and 28 dpi, respectively (Fig. 2A). Significant levels ($p < 0.0001$) of neutralizing antibodies were also observed, with gps exhibiting levels of 347 ± 65, 311 ± 92 and 325 ± 129 reciprocal dilutions at 0, 14 and 28 dpi, respectively (Fig. 2B).

An unprecedented number of local and international medical personnel in West Africa, Spain and the USA have been infected in the line of duty during the 2014 EBOV outbreak, despite wearing full protective gear. These developments have highlighted the urgent need for a vaccine so that they are able to work safely without putting their lives at risk. Evaluating the effectiveness of promising medical countermeasures against a more common mode of EBOV transmission (Dowell et al., 1999) is particularly relevant
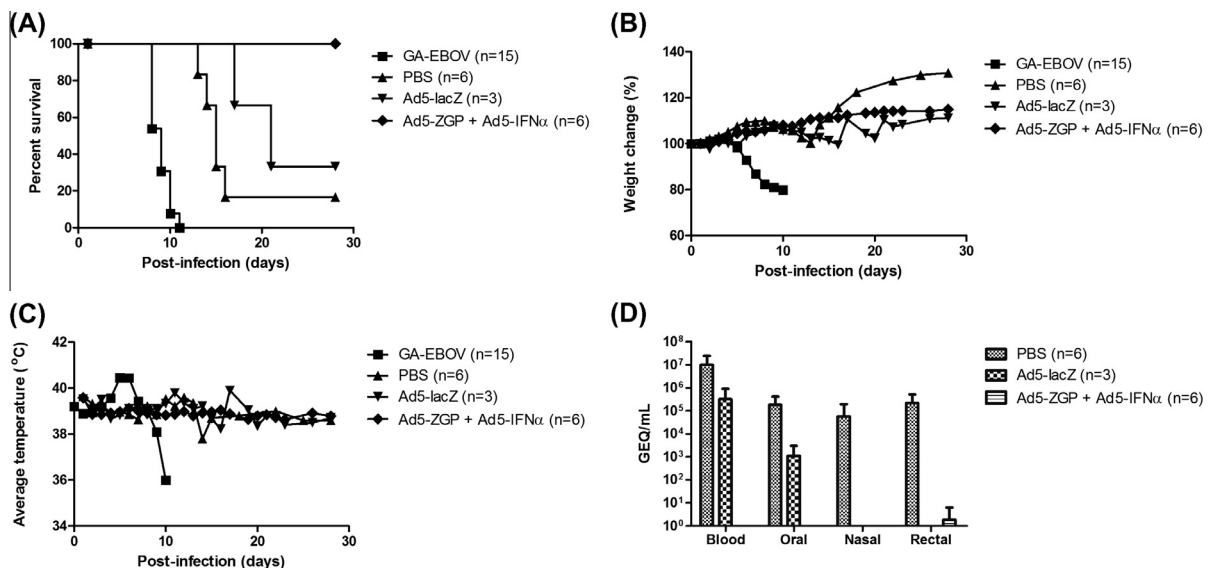

Fig. 1. Survival, weight loss, clinical symptoms and GA-EBOV shedding in infected and vaccinated contact gps. Challenge animals were intranasally (IN) infected with GA-EBOV and contact animals were administered PBS, Ad5-lacZ, or adjuvanted Ad5-ZGP via the IN route, 26 days before challenge animals were given GA-EBOV. All animals were monitored for changes in (A) survival, (B) weight loss and (C) body temperature for 4 weeks after infection. (D) Oral, nasal and rectal swabs were sampled from all animals at 14 dpi and quantitated for levels of virus shedding by RT-qPCR. RT-qPCR results were expressed in GEQ/mL. Error bars represent ± standard deviation.
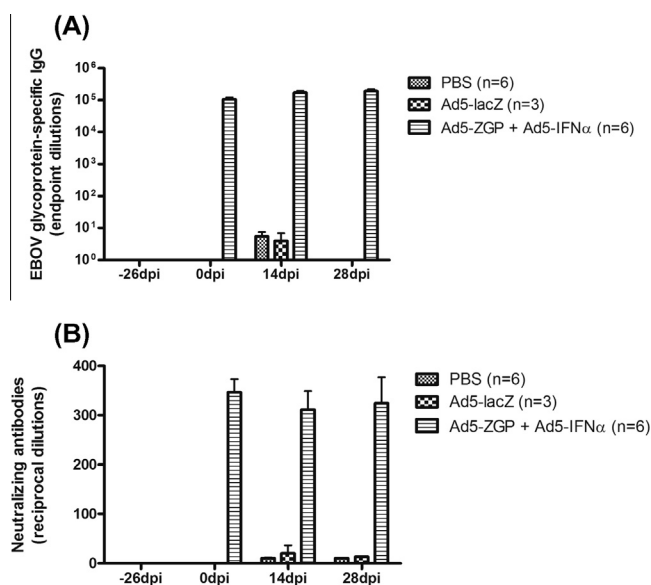
Fig. 2. Specific humoural immune responses in treated contact gps following vaccination and exposure to GA-EBOV infected animals. Guinea pigs were administered PBS, Ad5-lacZ, or adjuvanted Ad5-ZGP via the IN route and sera were sampled before vaccination (−26 dpi), before challenge (0 dpi), and then at 14 and 28 dpi. Levels of (A) ZGP-specific IgG antibodies and (B) EBOV-specific neutralizing antibodies were analyzed and reported as endpoint titers and reciprocal dilutions, respectively. Error bars represent ± standard deviation.

to the situation of front-line health care workers, as well as traditional burials or the reintroduction of a convalescent patient from quarantine back into the community. Immunization with Ad5 vaccines was performed IN for several reasons: (1) the gp transmission model used in this study supported GA-EBOV spread from infectious to naïve animals through the airway (Wong et al., 2014); (2) the relative ease of administration compared to IM injections; (3) IN administration of Ad5-ZGP had previously been shown to be efficacious in NHPs, even in the presence of pre-existing immunity toward the same adenovirus serotype (Richardson et al., 2013); and (4) the ability of mucosal Ad5-ZGP vaccination to induce durable protection against EBOV challenge in NHPs (Choi et al., 2014). Although the Ad5-ZGP vaccine is not currently under consideration for use against the 2014 EBOV outbreak in West Africa due to concerns over the prevalence of pre-existing immunity against Ad5 among humans in sub-Saharan Africa (Nwanegbo et al., 2004), these studies indicate that Ad5-based vaccines may still be beneficial if advanced into human use to combat any current or future EBOV outbreaks. Administration with the control Ad5-lacZ did not prevent GA-EBOV transmission and infection from contagious animals, as observed by the partial survival of contact gps in addition to the detection of viral RNA in the blood and shedding through oral swabs. In contrast, IN administration of adjuvanted Ad5-ZGP was effective in fully protecting gps from disease following prolonged exposure to an untreated, moribund animal. Clinical symptoms were not observed and GA-EBOV RNA was not detected in the secretions of vaccinated animals despite prolonged exposure with the contagious animal, suggesting that vaccination was effective in protecting the otherwise susceptible hosts from GA-EBOV transmission, and preventing the vaccine recipients from further propagating EBOV spread.

Although gps do not succumb to infection with wild-type EBOV and an adapted variant has been utilized in its place, the hallmarks

of GA-EBOV infection in gps are quite comparable to wild-type EBOV infection in NHPs. Taken together, IN vaccination with Ad5-based EBOV vaccines is an effective countermeasure against disease caused by EBOV spread between susceptible hosts and has the potential to prevent the occurrence of new infections during an EBOV outbreak.

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