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An Ebola whole-virus vaccine is protective in nonhuman primates

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Abstract

Zaire ebolavirus is the causative agent of the current outbreak of hemorrhagic fever disease in West Africa. Previously, we showed that a whole Ebola virus (EBOV) vaccine based on a replication-defective EBOV (EBOV VP30) protects immunized mice and guinea pigs against lethal challenge with rodent-adapted EBOV. Here, we demonstrate that EBOV VP30 protects nonhuman primates against lethal infection with EBOV. Although EBOV VP30 is replication-incompetent, we additionally inactivated the vaccine with hydrogen peroxide; the chemically inactivated vaccine remained antigenic and protective in nonhuman primates. EBOV VP30 thus represents a safe, efficacious, whole-EBOV vaccine candidate that differs from other EBOV vaccine platforms in that it presents all viral proteins and the viral RNA to the host immune system, which might contribute to protective immune responses.

The Ebola virus (EBOV) outbreak in West Africa has already claimed more than 5000 lives (1) and remains uncontrolled. One countermeasure to mitigate Ebola virus infections is vaccination. Several Ebola virus vaccine platforms have been developed over the last decades (2), three of which recently advanced to clinical trials: a DNA-based vaccine

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Tables S1 to S3

Figs. S1 and S2

expressing different Ebola virus glycoproteins (GPs, the major Ebola virus immunogen) (3, 4), a replication-incompetent chimpanzee adenovirus expressing GP (5), and a live-attenuated vesicular stomatitis virus (VSV) expressing GP (5). The DNA platform completely protects nonhuman primates (the “gold standard” for Ebola virus research) only after multiple dosages of the DNA vaccine in combination with recombinant adenovirus (6), but has not been tested as a standalone vaccination strategy. The recombinant adenovirus platform (including the recently developed recombinant chimpanzee adenovirus) requires high vaccine doses and boosting to achieve complete and durable protection of nonhuman primates against lethal challenge with EBOV (7, 8). Complete protection of nonhuman primates against lethal EBOV challenge has also been accomplished with the VSV platform; however, the use of a replicating recombinant VSV (9–12) may be of concern because of issues related to vaccine safety. Hence, although several platforms are being tested in clinical trials, additional options should be explored.

Whole-virus vaccines (either live attenuated or inactivated) have a long history as successful human vaccines, offering protection against potentially deadly viral diseases such as smallpox, influenza, mumps, and measles (13). Whole-virus vaccines present multiple viral proteins and the viral genetic material to the host immune system, which may trigger a broader and more robust immune response than vectored vaccines that present only single viral proteins. However, initial attempts to develop a gamma-irradiated, inactivated whole-EBOV vaccine failed to provide robust protection of nonhuman primates against challenge with a lethal dose of EBOV (14).

Previously, we developed a replication-defective EBOV (termed EBOV VP30) which is based on the Mayinga strain of EBOV and lacks the coding region for the essential viral transcription activator, VP30 (15). EBOV VP30 replicates to high titers in cell lines that stably express the VP30 protein, is genetically stable, and is nonpathogenic in rodents (15, 16). Mice and guinea pigs immunized twice with EBOV VP30 were fully protected against a lethal challenge with mouse- or guinea pig-adapted EBOV, respectively (16). EBOV VP30 is a biosafety level-3 agent and exempt from “Select Agent” status; an EBOV VP30 vaccine could therefore be manufactured in existing biosafety level-3 facilities that operate under good manufacturing practices.

To assess the effectiveness of EBOV VP30 whole-virus vaccine in nonhuman primates, we inoculated groups of cynomolgus macaques (Table 1) intramuscularly (i.m.) with Dulbecco’s modified essential medium (DMEM) (control, group 1), a single dose of 10^7 focus-forming units (FFU) of EBOV VP30 (group 2), or two doses of 10^7 FFU of EBOV VP30 4 weeks apart (group 3). Previously, we demonstrated the genomic stability of EBOV VP30 by carrying out three independent experiments that each comprised seven consecutive passages of the virus in VeroVP30 cells. After the last passages, we sequenced the region surrounding the VP30 deletion site and did not detect any recombination events or mutations. Moreover, the passaged viruses did not grow in wild-type cells, further indicating the lack of recombination. Despite these findings, concerns have been raised that such an event could potentially affect vaccine safety. Recently, virus inactivation with hydrogen peroxide was shown to preserve the antigenicity of lymphocytic choriomeningitis (17, 18), vaccinia (17), West Nile (17, 19), and influenza (20) viruses. To increase the

biosafety profile of EBOV VP30, we therefore treated it with hydrogen peroxide (H_2O_2 , 3% final concentration) for 4 hours on ice, followed by viral plaque assays in VP30-expressing cells, which confirmed complete virus inactivation. Nonhuman primates were then vaccinated twice with 10^7 FFU of the H_2O_2 -treated EBOV VP30 (group 4; two animals). Gamma-irradiation is an established procedure for Ebola virus inactivation, but irradiation conditions optimized for virus inactivation (rather than for antigenic epitope preservation) may alter antigenicity and therefore protective efficacy of Ebola virus vaccines (14). To test these concepts, we also vaccinated macaques twice with 10^7 FFU of wild-type EBOV gamma-irradiated in BSL-4 containment (group 5); again, the irradiation conditions used here ensured virus inactivation, but were not optimized to preserve antigenicity. None of the vaccinated animals showed signs of illness, confirming our earlier data from mice and guinea pigs that EBOV VP30 is nonpathogenic in animals (16).

Four weeks after the last immunization, we challenged animals in BSL-4 containment i.m. with a lethal dose (1000 FFU) of the heterologous Kikwit strain of EBOV. While control macaques in group 1 had to be euthanized on day 7 or 8 after challenge according to established and approved humane endpoint criteria (21) (Table 1), all animals immunized once (group 2) or twice (group 3) with the EBOV VP30 vaccine survived the lethal challenge (Table 1). In addition, both animals immunized twice with H_2O_2 -treated EBOV VP30 vaccine (group 4) survived infection with wild-type EBOV, indicating that H_2O_2 -treated EBOV VP30 is immunogenic and elicits protective immune responses. In contrast, all macaques immunized with gamma-irradiated wild-type EBOV (group 5) developed signs of severe EBOV disease and had to be euthanized between days 6 and 9 after challenge (Table 1), supporting the concept that gamma-irradiation optimized for virus inactivation alters the immunogenicity of EBOV vaccines. The macaques that had to be euthanized after challenge with EBOV (groups 1 and 5) had high virus titers in their blood after challenge (Fig. 1). In contrast, no viremia was detected in animals immunized twice with untreated (group 3) or H_2O_2 -treated EBOV VP30 (group 4) (Fig. 1), showing that H_2O_2 -treated EBOV VP30 elicited a protective immune response. One of four animals that received a single immunization with EBOV VP30 [nonhuman primate (NHP) 8 in group 2] was viremic on days 3 and 6 after challenge, but cleared the virus on day 9 (Fig. 1). In addition, a different animal in group 2 (NHP 7) had a fever on day 6 after challenge (table S1). These data indicate that a single vaccination with EBOV VP30 does not always prevent EBOV replication or signs of illness (fever), but does protect the host from death upon EBOV challenge. Together, our findings demonstrate the vaccine potential of a whole-EBOV vaccine based on EBOV VP30.

To better understand the correlates of protection, we measured the immune responses 2 and 4 weeks after the last immunization (i.e., 2 weeks and immediately before EBOV challenge). Two weeks after the last vaccination (day -14), macaques immunized twice with EBOV VP30 (group 3) had a high immunoglobulin G (IgG) antibody response to the viral GP based on a GP-specific enzyme-linked immunosorbent assay (ELISA) assay (Fig. 2). Two immunizations with H_2O_2 -treated EBOV VP30 (group 4) resulted in a slightly lower, but still robust, immune response (Fig. 2). In macaques immunized once with EBOV VP30 (group 2), we detected a low, but measurable, IgG antibody response (Fig. 2). Serum samples from animals that succumbed to EBOV challenge—namely, those mock-

immunized (group 1) or immunized twice with gamma-irradiated wildtype EBOV (group 5) —did not possess measurable IgG titers to GP (Fig. 2). The IgG titers to EBOV GP on the day of challenge (day 0, Fig. 2) followed the same trend, but were low. The IgG titers to EBOV GP closely mirrored survival rates and virus titers (Table 1 and Fig. 1); these data indicate that immunization with EBOV VP30 elicits an antibody response to GP that is important for protection against EBOV infection. A similar correlation between a GP-specific antibody response and protection has been demonstrated with other experimental EBOV vaccine platforms (22, 23).

The antibody repertoire was further characterized by assessing the levels of neutralizing antibodies to GP as measured by plaque reduction neutralization (PRNT) assays. The serum dilution that reduced the titer of VSV-expressing EBOV GP by 50% (plaque reduction neutralization titer 50, PRNT₅₀) was 1:20 to 1:40 for samples obtained from animals immunized twice with EBOV VP30 (group 3; table S2); no statistically significant decline in neutralizing antibody levels was detected between day -14 (2 weeks before challenge) and day 0 (table S2). In contrast, we detected slightly lower PRNT₅₀ titers of ~1:10 for sera obtained from animals immunized once with untreated or H₂O₂-treated EBOV VP30 (groups 2 or 4, respectively; table S2). No neutralizing antibodies were detected in control animals or animals immunized twice with gamma-irradiated EBOV (groups 1 or 5, respectively; table S2). Overall, the neutralizing antibody titers were low, but similar to those detected upon vaccination of animals with VSV-expressing EBOV GP (11).

Most experimental Ebola virus vaccine platforms provide only the viral GP as antigen, expressed from recombinant viruses or protein expression plasmids; in contrast, the EBOV VP30 vaccine presents all viral proteins plus the viral genetic material to the host. Early studies with EBOV-like particles (VLPs) suggested that the viral matrix protein (VP40) and nucleoprotein (NP) are also immunogenic (24), prompting us to carry out ELISAs specific for these two viral proteins. Two weeks after the last vaccination (day -14), macaques immunized twice with untreated (group 3) or H₂O₂-treated (group 4) EBOV VP30 had high NP and VP40 antibody titers (fig. S1). Lower, but still robust, NP and VP40 antibody titers were observed in macaques immunized once with EBOV VP30 (group 1). Contrary to the GP antibody titers, we also detected NP and VP40 antibodies in animals immunized twice with gamma-irradiated EBOV (group 5), suggesting that gamma-irradiation under conditions optimized for virus inactivation has a greater effect on the antigenicity of GP epitopes than on that of NP and VP40 epitopes. Collectively, these data demonstrate that antibodies to NP and VP40 are elicited after vaccination with EBOV VP30 and that the levels of these antibodies are higher in protected animals than in those that succumbed to infection. However, the importance of NP and VP40 antibodies to protection from EBOV infection is not yet known.

In addition to the antibody response, we also measured the cellular immune response by examining the number of mononuclear cells producing interferon- γ (IFN- γ). On day -14 (2 weeks before challenge), animals in groups 2 and 3, immunized one or twice with EBOV VP30, respectively, had the highest number of IFN- γ -producing cells (fig. S2). Although treatment of EBOV VP30 with H₂O₂ (group 4) reduced the number of IFN- γ -producing cells, more IFN- γ -producing cells were detected in these animals compared with

those immunized twice with gamma-irradiated EBOV (group 5; fig. S2) or left untreated (group 1; fig. S2).

Data from Geisbert *et al.* (14) and our present findings suggest that gamma-irradiation optimized to inactivate EBOV destroys the antigenicity of wild-type EBOV, particularly in EBOV GP. H₂O₂-treated EBOV VP30, however, elicited a robust IgG response and protected nonhuman primates against lethal EBOV challenge, although H₂O₂ treatment resulted in a slight reduction of antigenicity compared with untreated virus (Fig. 2). Hence, H₂O₂ treatment of EBOV VP30 appears to preserve key antigenic epitopes, as has been demonstrated for other viruses (17–20). To examine potential differences in antigenicity between gamma-irradiated and H₂O₂-treated virus, we performed an ELISA-based assay, using a panel of 19 monoclonal antibodies (mAbs) directed against GP. Most mAbs showed levels of binding comparable to that of GP; however, four (mAbs 12, 21, 226, and 662) reacted more efficiently with H₂O₂-treated than with gamma-irradiated virus (Fig. 3). Most likely, gamma-irradiation affected the conformation of the epitopes recognized by these antibodies, resulting in the lack of protection upon immunization with gamma-irradiated virus. Hence, the epitopes recognized by mAbs 12, 21, 226, and 662 may play an important role in antibody-mediated protection in immunized macaques and potentially in humans; indeed, mAb 226 is known to have virus-neutralizing properties (25). OnemAb (1031) interacted more efficiently with gamma-irradiated than with H₂O₂-treated virus, while a polyclonal antiserum reacted similarly with both virus preparations tested (Fig. 3).

When EBOV was first discovered over 35 years ago, whole-virus vaccines inactivated by formalin or gamma-irradiation were tested, but failed to elicit complete protection in nonhuman primates (14). The development of whole-virus vaccines was therefore abandoned, and VLPs composed of GP and VP40 (and NP) were explored as a safe and immunogenic platform to present several viral proteins to the host immune system (2, 26–28). These VLPs are immunogenic, but three vaccinations with adjuvanted VLPs were required to achieve protective efficacy in nonhuman primates (24). Here, we present a vaccine strategy that offers several advantages: (i) It provides protection from a lethal challenge of EBOV in nonhuman primates after a single immunization, although one animal became viremic and another animal developed a fever; (ii) it is highly immunogenic, as shown by robust antibody responses elicited upon vaccination; (iii) it is amenable to largescale production, because EBOV VP30 grows to titers of $>10^7$ FFU/ml in VP30-expressing cells (15); (iv) it is safe, owing to its inability to replicate outside VP30-expressing cells (15); and (v) it presents all viral proteins and its genomic RNA to the host, similar to whole-virus vaccines and VLPs. It should be noted that NHPs immunized once with EBOV VP30 (group 2) were protected from a lethal EBOV challenge, although two of the four animals showed signs of illness (fever was detected in NHP 7, and viremia was detected in NHP 8; table S1). However, all four animals in group 2 (NHPs 5 to 8) showed similar immune responses (table S2 and summarized in table S3).

To address any potential concerns over recombination events that would restore the replicative ability of EBOV VP30, we also chemically inactivated it with H₂O₂. Hydrogen peroxide treatment causes breaks in single- and double-stranded DNA or RNA (17) and thus inactivates viruses without affecting their antigenicity. By contrast, gamma-irradiation (used

to generate the first experimental whole EBOV vaccine) causes the (de) hydroxylation of amino acids, the cleavage of polypeptide backbones (29), and the generation of free radicals that could cause the destruction of the antigenic properties of some epitopes. These differences in mechanism may explain why viruses treated with H₂O₂ are more immunogenic than those irradiated with gamma rays; however, optimization of irradiation conditions may improve the immunogenicity of vaccine candidates.

In summary, our data indicate that EBOV VP30 is an effective whole-EBOV vaccine that warrants further assessment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Virus titers in the blood of infected nonhuman primates

Animals were immunized as shown in Table 1. Four weeks after the last immunization, animals were infected with a lethal dose of EBOV. Shown are EBOV titers in the blood of individual nonhuman primates from each group. Virus titers are shown as 50% tissue culture infective dose (TCID₅₀).

Fig. 2. Immune responses in vaccinated nonhuman primates

IgG antibody responses to EBOV GP 2 weeks after the last vaccination (day -14) and on the day of challenge (day 0). Antibody titers were measured with an ELISA specific for EBOV GP. Titers shown are the highest reciprocal dilution that resulted in an optical density (OD) of 0.2.

Fig. 3. Effects of H₂O₂-treatment and gamma-irradiation on the antigenicity of EBOV GP

Using a panel of 19mAbs (1 µg/ml) directed against EBOV GP, we performed an ELISA to examine the antigenicity of gamma-irradiated EBOV (blue) and H₂O₂-treated EBOV VP30 (red).

Table 1

Overview of vaccination and challenge strategy.

Group	Vaccine	Inactivation	Vaccination		Protection	Euthanasia
			Prime	Boost		
Group 1	Mock [*]	—	—	—	0% [‡] (<i>n</i> = 4)	Days 7 to 8 [§]
Group 2	EBOV VP30	—	1 × 10 ⁷ FFU	—	100% (<i>n</i> = 4)	N/A [§]
Group 3	EBOV VP30	—	1 × 10 ⁷ FFU	1 × 10 ⁷ FFU	100% (<i>n</i> = 4)	N/A
Group 4	EBOV VP30	Hydrogen peroxide	1 × 10 ⁷ FFU	1 × 10 ⁷ FFU	100% (<i>n</i> = 2)	N/A
Group 5	EBOV	Gamma-irradiation	1 × 10 ⁷ FFU	1 × 10 ⁷ FFU	0% (<i>n</i> = 4)	Days 6 to 9

* DMEM.

[‡] Percentage of animals that survived challenge with a lethal dose of EBOV.

[§] Days after challenge.

[§] N/A, not applicable.