Review

Ebolavirus Vaccines: Progress in the Fight Against Ebola Virus Disease

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Key Words

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Abstract

Ebolaviruses are highly infectious pathogens that cause lethal Ebola virus disease (EVD) in humans and non-human primates (NHPs). Due to their high pathogenicity and transmissibility, as well as the potential to be misused as a bioterrorism agent, ebolaviruses would threaten the health of global populations if not controlled. In this review, we describe the origin and structure of ebolaviruses and the development of vaccines from the beginning of the 1980s, including conventional ebolavirus vaccines, DNA vaccines, Ebola virus-like particles (VLPs), vaccinia virus-based vaccines, Venezuelan equine encephalitis virus (VEEV)-like replicon particles, Kunjin virus-based vaccine, recombinant Zaire EbolavirusΔVP30, recombinant cytomegalovirus (CMV)-based vaccines, recombinant rabies virus (RABV)-based vaccines, recombinant paramyxovirus-based vaccines, adenovirus-based vaccines and vesicular stomatitis virus (VSV)-based vaccines. No licensed vaccine or specific treatment is currently available to counteract ebolavirus infection, although DNA plasmids and several viral vector approaches have been evaluated as promising vaccine platforms. These vaccine candidates have been confirmed to be successful in protecting NHPs against lethal infection. Moreover, these vaccine candidates were successfully advanced to clinical trials. The present review provides an update of the current research on Ebola vaccines, with the aim of providing an overview on current prospects in the fight against EVD.

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Introduction

Ebolavirus was first recognized during synchronous outbreaks of hemorrhagic fever in Zaire and Sudan in 1976 [1]. Since then, several clusters of ebolavirus infections have emerged in Africa. The five currently known species of ebolaviruses, in the order of their time of identification, are Zaire ebolavirus (EBOV) [2], Sudan ebolavirus (SUDV) [3], Tai Forest X.-X. Wu and H.-P. Yao contributed equally to this article.

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ebolavirus (TAFV) [4], Reston ebolavirus (RESTV) [5] and Bundibugyo ebolavirus (BDBV) [6, 7]. In 2014, ebolavirus infections shifted to Western Africa, causing high morbidity and mortality. By August 19th, 2015, the number of cases reached 27988, with more than 11299 deaths [8].

Ebolavirus, as a category A pathogen, causes a highly lethal hemorrhagic fever [9]. Due to frequent travel between different countries for activities such as trading, tourism and medical assistance, the risk of global transmission of ebolavirus is ever present. Recent reports of Ebola cases in the U.S. and Spain have reminded the world that Ebola virus disease (EVD) is no longer a regional disease [10, 11]. Furthermore, it has greatly impacted public health systems by causing numerous deaths of healthcare workers and health facility closures. The potential misuse of ebolaviruses as bioterrorism agents may lead to a public safety crisis given the current lack of specific treatment and vaccination methods [12].

For many infectious diseases, the patient's immune system can eventually control the pathogen, but its ability to mount an effective response to ebolavirus is difficult. The rapid depletion of lymphocytes by apoptosis is a prominent feature of the disease, resulting in the impairment of host immune responses [13]. Virus-induced expression of inflammatory mediators causes an immunological imbalance and impairment of the vascular system. Coagulation abnormalities together with hypotension resulting from ebolavirus infection cause patients to rapidly enter into systemic shock and multi-organ failure [9]. In some cases, patients die due to the inability to generate an effective immune response.

Although no specific treatment for EVD has been approved, ZMapp, a combination of three humanized monoclonal antibodies, has shown protection in non-human primates (NHPs), and some studies [14-16] have demonstrated that vaccines based on targets of these antibodies can be successfully generated. Fortunately, significant progress in ebolavirus vaccine development has been made in recent years, resulting in at least six promising vaccine candidates (Table 1). This review provides an overview of the known structure of ebolavirus and the history of ebolavirus vaccine research.

Structure of ebolavirus

Ebolavirus is a member of the family of filoviruses, which are enveloped, non-segmented, negative-strand RNA viruses [17]. Ebolavirus particles have three basic morphological forms: empty, linked and continuous. They have a uniform diameter of approximately 80 nm, and the most common class length of virus particles is 982 ± 79 nm [17]. The ebolavirus genome is comprised of seven non-segmented genes (Fig. 1), which encode (from the 3' leader to the 5' trailer) the nucleoprotein (NP), virion protein (VP) 35, VP40, glycoprotein (GP), VP30, VP24 and RNA-dependent RNA polymerase (L) [18].

All ebolavirus genes each encode one structural protein, except the GP gene which encodes secreted GP and GP. Secreted GP, as the primary product of the GP gene, has the ability to interfere with the host immune response. GP has two domains including GP1 and GP2. GP1 contains the receptor binding domain, and GP2 contains the fusion and transmembrane domains [17]. GP is the unique transmembrane surface protein mediating virus entry into the host cell after binding with its receptor. Meanwhile, it can cause vascular leakage through interactions with endothelial cells. VP40, as the matrix protein, mediates particle formation in the host cell [19]. VP24 is another structural protein associated with the membrane and interferes with interferon signaling [20]. The RNA genome is encapsulated by the nucleoprotein to form the inner ribonucleoprotein complex. The nucleoprotein associates with VP35, VP30 and RNA-dependent RNA polymerase, forming the functional transcriptase-replicase complex. VP35 alone has the additional role of an interferon antagonist [21]. The nucleocapsid consists of VP24, VP30, NP and VP35. VP24 and VP35 form the bridge located on the periphery of the nucleocapsid [17]. Among these viral proteins, GP, NP and VP40 are typically used to develop vaccines (Fig. 1).

Vaccine vector	Immunogen	Evaluation NHPs	Advantages in	Clinical trial	Main concerns	Ref
DNA	GP; NP	100% protection	Inexpensive, safe, stable	Phase 1 clinical trial	Boost immunization	[36-41]
Ad5	GP; NP; GP/NP	100% protection	Cross-protection, multiple routes of immunization	Phase 1 clinical trial	Pre-existing immunity; Toxicity in large dose	[83-85,97,98]
Ad26	GP	100% protection	Low serum prevalence in humans	Phase 3 clinical trial	Toxicity in large dose	[54,99]
MVA-BN	GP	100% protection	Favorable safety ; Robust	Phase 3 clinical trial	Boost immunization	[54]
ChAd3	GP;	100% protection	No pre-existing immunity	Phase 3 clinical trial	Safety	[101-103]
VSV	GP	100% protection	Post-exposure Cross-protection	Effective in one case; Phase 3 clinical trial finished	Safety	[119-123]

Table 1. Current promising ebolavirus vaccine candidates. NOTE: NHPs, non-human primates; Ref, references; Ad5, adenovirus 5; Ad26, adenovirus 26; MVA-BN, Modified Vaccinia Ankara-Bavarian Nordic; ChAd3, Chimpanzee adenovirus type 3 ;VSV, vesicular stomatitis virus; GP, glycoprotein; NP, nucleoprotein

Ebolavirus vaccines

Ebolavirus vaccines have been investigated since the first known outbreak of EVD. The first ebolavirus vaccine produced was an inactivated virus vaccine, which was tested for efficacy in guinea pigs in 1980. In the following years, an increasing number of ebolavirus vaccines have been developed. The sequence of key events in the development of ebolavirus vaccines is provided in Fig. 2 (timeline), including conventional vaccines, DNA vaccines, Ebola virus-like particles (VLPs) and vector-based vaccines.

Conventional ebolavirus vaccines

Conventional ebolavirus vaccines can be generated through inactivation of the virus by heat, formalin or γ-irradiation [22]. In the early 1980s, the first successful attempt using inactivated virus showed protection of guinea pigs from challenge with EBOV [23], but another study produced the opposite result [24]. Later, Matyas et al. used liposome-encapsulated irradiated EBOV to immunize mice and found that it could completely protect against illness and death [25]. However, further research showed that conventional vaccines failed to successfully protect NHPs from robust challenge with EBOV [26]. Furthermore, retention of virulence of mouse-adapted and guinea pig-adapted EBOV in NHPs contributed to safety concerns [27]. Fortunately, modern genetic engineering techniques have provided us with new approaches to improving ebolavirus vaccine candidates.

DNA vaccines

DNA vaccines are plasmids that have been genetically engineered to produce one or two targeted proteins from a pathogen [28] and have virtually no risk of infection. Additionally, they are easily developed, stable and inexpensive to produce [29]. Furthermore, DNA vaccines do not require the complicated manufacturing process of recombinant proteins or the use of toxic adjuvants [30].

Fig. 1. Structure of ebolavirus and ebolavirus vaccine vectors. Shown are schematic diagrams of ebolavirus (center) and vaccine vectors, including inactivated ebolavirus vaccine, DNA vaccine, VLPs, VEEV NP-VPR, VEEV GP-VPR, recombinant EBOVΔVP30, rHPiV3, rAd5 and rVSV. Ebolavirus genes and proteins are shown in different colors (red: nucleocapsid proteins, yellow: matrix proteins, blue: glycoprotein). Genes and proteins of the vaccine vectors are shown in pink. For rVSV, G was deleted and replaced with GP in the genome of VSV. For rAd5, genome deletions in the E1 and E3 regions, as well as the GP insertion, are indicated. For rHPiV3, G was deleted and replaced with GP in the genome of HPiV3. NP-VPR, NP-replicon particles; GP-VPR, GP-replicon particles; VEEV, Venezuelan equine encephalitis virus; rHPiV3, recombinant human parainfluenza virus type 3; rAd5, recombinant adenovirus 5; rVSV, recombinant vesicular stomatitis virus.

Ebola DNA vaccines mainly have been constructed to express the GP or NP gene. Four doses of a GP DNA vaccine was shown to completely protect mice from lethal ebolavirus challenge [31]. Multi-targeting DNA vaccines expressing GP genes of EBOV, SUDV and Marburg virus were demonstrated to be effective in mice [32]. DNA vaccines were also effective when evaluated in a guinea pig infection model [33-35]. Furthermore, DNA immunization and boosting with adenoviral vectors that encode GP were shown to protect NHPs from lethal EBOV challenge, while inducing both cellular and humoral immunity [36]. Another report showed that DNA plasmids expressing codon-optimized GP genes of EBOV provided good protection to cynomolgus macaques against EBOV challenge.

Taken together, evaluations of DNA vaccines in rodents and NHPs have shown promise for their use in humans. The DNA vaccine made using transmembrane-deleted and point-mutation EBOV GP was evaluated in a clinical trial in 2006 and found to be well-tolerated and produced no significant adverse events or coagulation abnormalities [37]. Moreover, specific antibodies as well as T-cell responses were detected in humans after three immunizations with this DNA vaccine [38]. After the ebolavirus epidemic in Western Africa of 2014, DNA vaccines were evaluated again for this disease. Wild-type GP from EBOV or SUDV was used to generate DNA vaccines, which were quickly tested in clinical trials. These DNA vaccines were given (4 mg administered intramuscularly by Biojector) at weeks 0, 4 and 8, with a homologous boost at or after week 32. The DNA vaccines were validated to be safe, well-tolerated and immunogenic in the Phase 1 study [39]. A subsequent Phase 1b, double-blinded, randomized, placebo-controlled clinical trial was carried out in Kampala, Uganda to

Fig. 2. Timeline/key events in ebolavirus vaccine development. Vaccines which tested in clinical trials are boxed in red. EBOV, Zaire ebolavirus; VSV, vesicular stomatitis virus; VEEV, Venezuelan equine encephalitis virus; HPIV3, human parainfluenza virus type 3; rEBOVΔVP30, recombinant EBOV with viral protein 30 deletion; Ad5, adenovirus 5; CMV, cytomegalovirus; RABV, rabies virus; VLPs, virus like particles; Ad26-EBOV, adenovirus 26 loaded with EBOV GP gene; rVSV-EBOV, recombinant vesicular stomatitis virus vaccine; ChAd3-EBOV, chimpanzee-derived adenovirus 3-vectored ebolavirus vaccine. MVA-BN filo, Modified Vaccinia Ankara-Bavarian Nordi-vectored ebolavirus vaccine.

examine the safety and immunogenicity of the DNA vaccines, and both of them were deemed safe and elicited antigen-specific humoral and cellular immune responses [40, 41].

Ebola VLPs

VLPs have relatively low biosafety concerns due to their lack of a viral genome, and their use can bypass issues associated with pre-existing immunity [42]. Ebola VLP vaccine candidates can be generated by the expression of VP40 alone or along with GP and NP [43]. Ebolavirus VP40 is sufficient for virus assembly and budding from the plasma membrane [44]. The interaction between VP40 and GP is significant in virus morphogenesis, which results in spontaneous production and release of Ebola VLPs [18].

In recent years, Ebola VLPs have been tested in mice, guinea pigs and NHPs against lethal challenge with EBOV [45, 46]. Ebola VLPs were effective in raising the numbers of natural killer (NK) cells in lymphoid tissues [47], and co-administration with QS-21 adjuvant could improve the immunogenicity of Ebola VLPs. CD8⁺ T cells were demonstrated to be absolutely required for Ebola VLP-mediated protection against EBOV infection [48]. A multivalent VLP vaccine expressing heterologous viral proteins of EBOV and Marburg virus elicited strong immune responses and protected guinea pigs against challenge with both of these viruses [49]. In another study, all Ebola VLP-vaccinated NHPs survived EBOV challenge without clinical or laboratory signs of infection [50].

Ebola VLPs have been most commonly produced through transient transfection of mammalian cells. Unfortunately, this process is expensive, time-consuming and labor-intensive. For large-scale production, insect cell lines were developed, and insect cell-

derived Ebola VLPs were shown to be highly immunogenic and effective vaccines for the prevention of Ebola infection [51]. The ecdysone-inducible mammalian expression system was also developed to create stable cell lines expressing Ebola VLPs [52]. Ebola VLPs are also promising as a prophylactic vaccine, and greater effort should be made to increase their production and provide coverage for all ebolavirus species.

Vector-based vaccines

Multiple viruses have been used as vaccine vectors, which can be replication competent or defective. Replication-defective viral vectors are safe but require multiple doses to achieve optimal immunity. While replication-competent vectors generally induce strong and long-lasting immune responses, they are not suitable for immune-compromised individuals. In the following sections, several vector-based vaccines will be discussed.

Vaccinia virus-based vaccine

The first vaccinia virus-based vaccine, which was constructed to express ebolavirus VP24, was shown to prolong the mean lifespan of guinea pigs challenged by EBOV [53]. Later, a vaccinia virus-based vaccine expressing GP was tested in cynomolgus macaques but unfortunately did not protect the animals against lethal homologous challenge, even though neutralizing antibodies against EBOV were detected [26]. By the end of 2014, a novel MVA-BN filo vaccine was produced by the Bavarian Nordic Company. This vaccine was based on the modified vaccinia ankara (MVA) virus which is a robust and adaptable platform suitable for expressing genes of a filamentous virus. The MVA-BN filo vaccine was designed to protect against EBOV, SUDV and Marburg virus. Preclinical studies conducted by the United States National Institutes of Health demonstrated that a prime-boost vaccination regimen consisting of MVA-BN filo and Janssen's Ad26 EBOV vaccine resulted in complete protection from challenge with EBOV. The MVA-BN filo vaccine was soon entered into a Phase 1 trial and in preliminary results demonstrated good safety and immunogenicity. Moreover, the Phase 3 study using the Ad26 EBOV vaccine combined with the MVA-BN filo vaccine is currently ongoing. Another clinical trial using the combination of the cAd3-EBOV vaccine and MVA-BN filo vaccine was also conducted [54].

Venezuelan equine encephalitis virus (VEEV)-like replicon particles

VEEV is a positive-sense RNA virus [21]. By replacement of VEEV structural protein genes, the Ebola NP or GP gene was successfully packaged into recombinant VEEV replicon particles (VRP) and designated as NP-VRP or GP-VRP. These single-cycle replicons were propagation-deficient. The GP-VRP alone, or in combination with NP-VRP, was shown to protect both guinea pigs and BALB/c mice [55]. Another study also demonstrated the protective effects of NP-VRP in mice [56]. CD8⁺ cytotoxic T cells have been found to play a significant role in the protection against ebolavirus infection [57]. While the efficacy of vaccines observed in rodents does not always extend to primates [26], GP-VRP was evaluated in cynomolgus macaques in 2013 and found that a single dose could provide complete protection against intramuscular challenge [58].

Kunjin virus-based vaccines

Kunjin virus is an Australian subtype of West Nile virus belonging to the Flaviviridae family. Reynard et al. developed a Kunjin virus-based vaccine expressing ebolavirus GP,

membrane anchor-truncated GP and D637L-mutated GP [59]. The surviving animals showed complete clearance of the virus, but the anti-GP-specific antibody response was found to be variable between them. Thus, KUN replicons have the potential to be vaccine candidates, but additional studies are needed to identify the mechanism of the protective effect.

Recombinant EBOVΔVP30

Recombinant EBOVΔVP30 (rEBOVΔVP30) was developed using reverse genetics to artificially delete VP30 from the genome of EBOV. In 2008, Halfmann et al. first reported on the potential of rEBOVΔVP30 as a vaccine candidate [60]. rEBOVΔVP30 was shown to be unable to replicate and produce progeny unless provided with VP30 in trans. rEBOVΔVP30 particles were indistinguishable in morphology from wild-type virus and confirmed to be genetically stable. rEBOVΔVP30 was determined to be safe even in STAT-1 knockout mice, and its protective efficacy was evaluated in mice and guinea pigs. However, the safety and efficacy of rEBOVΔVP30 have not been evaluated in NHPs [61].

As a potential vaccine candidate, the possibility of reversion of rEBOVΔVP30 back to wild-type EBOV remains a safety concern. Fortunately, no evidence of re-integration of VP30 into the viral genome was observed during serial passaging of rEBOVΔVP30 in Vero cells expressing VP30 [61], but additional experiments should be conducted to further confirm its safety for use as a vaccine. In April 2015, rEBOVΔVP30 was reported to protect NHPs against lethal infection with EBOV when given either one or two doses, with each dose at 10⁷ focus-forming units (FFU). In order to increase the safety of rEBOVΔVP30, the vaccine was inactivated by hydrogen peroxide. Fortunately, the chemically inactivated vaccine remained antigenic and showed protection in NHPs [62]. The rEBOVΔVP30 vaccine presents all viral proteins and the viral RNA to the host, which would be expected to facilitate induction of protective immune responses, but future clinical trials are needed to validate its efficacy.

Recombinant cytomegalovirus (CMV)-based vaccines

CMV has been used as a platform for cancer and tetanus vaccines [63-65]. CMV-based vectors can achieve high vaccine coverage in inaccessible wildlife populations such as great apes due to its unique potential to re-infect and disseminate through target populations regardless of prior CMV immunity [66]. A recombinant mouse CMV (MCMV) vector, constructed to express a CD8⁺ T cell epitope from EBOV NP (43-VYQVNNLEEIC-53) and fused to a non-essential MCMV protein, was shown to protect C57BL/6 mice from challenge with a lethal dose of mouse-adapted EBOV [66]. A high level of long lasting (>8 months) CD8⁺ T cells against EBOV NP was generated in the mice, while a low level of anti-EBOV antibodies was sporadically detected [66]. Thus, the protective efficacy of CMV-based vaccines should be evaluated in larger animal models such as NHPs.

Recombinant rabies virus (RABV)-based vaccines

RABV is a member of the Rhabdoviridae family with a non-segmented, negative-strand RNA. Live-attenuated RABV has been demonstrated to be an excellent vaccine vector [67, 68]. As RABV causes more than 24,000 deaths per year in Africa [69], an effective bivalent RABV/ebolavirus vaccine would be a valuable public health tool in that region.

Inactivated and live-attenuated bivalent vaccines expressing EBOV GP based on the SAD B19 strain of RABV have been developed. The vaccine candidates were shown to be avirulent in adult mice and displayed low neurovirulence in suckling mice [70]. These vaccines also could induce humoral immunity and conferred protection from both RABV and EBOV lethal challenge in mice [71]. Furthermore, pre-existing immunity against RABV did not impact the

immunogenicity of these vaccines in mice [72]. Meanwhile, a replication-competent vaccine provided 100% protection against EBOV challenge, which was a better result than that achieved by the other two RABV vaccines [73]. Detailed investigation of adaptive immune responses showed that the successful ebolavirus vaccine could induce strong virus-specific antibodies [73]. However, more safety tests should be conducted before advancing the RABV-based vaccine to clinical trials.

Recombinant paramyxovirus-based vaccines

Human parainfluenza virus type 3 (HPIV3) has been explored as a vector for vaccination via the respiratory route. A vaccine was developed based on the replication-competent HPIV3 expressing ebolavirus GP (HPIV3/EboGP) and demonstrated to be feasible for intranasal immunization in a guinea pig model [74]. The effectiveness of HPIV3/EboGP also was evaluated in rhesus monkeys using the novel immunization method via the respiratory tract against EVD [75].

However, pre-existing immunity to HPIV3 in the adult human population may greatly impact the replication and immunogenicity of the vaccine in this population. An epidemiological investigation conducted in Japan showed the percentage of the human population that would have pre-existing immunity to HPIV3 is higher than 94% [76]. This restriction appeared to be based on both humoral and cellular immunity to HPIV3 [77]. The replication of HPIV3/EboGP was found to be highly restricted in HPIV3-immune animals as well [77]. Due to pre-existing immunity to HPIV3, a single dose of HPIV3/EboGP failed to prevent infection of HPIV3-immune monkeys, but two doses did provide complete protection [78]. Those results suggested that successful vaccination with HPIV3/EboGP could be achieved by increasing the number of doses in HPIV3-immune populations. In order to bypass the main obstacle of pre-existing immunity in humans, a Newcastle disease virus (NDV) vaccine candidate (NDV/GP) was developed. Evaluation of NDV/GP in rhesus monkeys suggested that it would be effective for immunization against ebolavirus [79].

Adenovirus-based vaccines

Recombinant adenovirus 5 (rAd5) vaccines were initially used for boost immunizations after priming with DNA vaccines [36], and protection was shown to be highly effective and dependent on Ebola-specific CD8⁺ T-cell and antibody responses [80]. CD8⁺ cells were found to play a major role in rAd5-GP-induced immune protection against EBOV infection in NHPs [81]. In another study using NHPs, the level of Zaire GP-specific IgG showed a meaningful correlation with the protection against EBOV exposure [82]. Moreover, rAd5 was found to be safe in a Phase I clinical trial, and the volunteers all developed antigen-specific humoral and cellular immune responses [83, 84].

Determining the safe dose of any adenovirus-based vaccine is necessary since high numbers of adenovirus particles (1×10^{13}) can be toxic to NHPs and humans [85]. The Ad5- and Ad35-based vaccines given intramuscularly at doses up to 2×10^{11} were shown to be safe and suitable for human use [86]. Furthermore, rAd5 expressing GP alone was sufficient to confer protection against lethal challenge with the Kitwit strain of ebolavirus in NHPs, and the minimal effective rAd dose was established at 10^{10} particles [87]. Attempts to lower this dose by using optimized expression cassettes for the immunogen were successful in mice, but they have not yet been evaluated in NHPs models.

Other attempts have been made to enhance the protective effects of ebolavirus vaccines. A bivalent rAd5 vaccine (cAdVax) expressing the SUDV GP and EBOV GP genes together was determined to be effective in mice [88]. The vaccine was also successfully used in NHPs to protect against the parenteral and aerosol routes of lethal challenge [89]. Another research team developed a rAd5-based Ebola vaccine expressing the EBOV GP sequence from a CMV

promoter (Ad-CMVZGP), which showed effective protection in mice, guinea pigs and NHPs against lethal challenge by EBOV [90].

A potential challenge for vaccine development is the continuing evolution of ebolaviruses. Therefore, a vaccine candidate with sufficient breadth to be used in response to new outbreaks of previously undetected viruses is greatly needed. DNA/rAd5 vaccines expressing EBOV and SUDV GP were demonstrated to protect NHPs against lethal challenge with BDBV and indicated that Ebola vaccines capable of eliciting potent cellular immunity may provide cross-protection against newly emerging heterologous ebolavirus species [91].

The original recombinant adenovirus vaccine was mainly based on Ad5. However, the considerable problem of pre-existing immunity severely compromised the efficacy of the human Ad5 vaccine [92]. A cross-sectional serological survey conducted in Brazil (Rio de Janeiro and São Paulo), Thailand (Bangkok), South Africa (Soweto), Malawi (Thyolo), Botswana (Gaborone) and Cameroon (Yaounde) showed that 85.2% of participants were positive for Ad5 [93]. Although high doses of adenovirus-based vaccines may override issues related to pre-existing immunity, the concern of toxicity due to the high number of adenovirus particles (1×10^{13}) in NHPs and humans remains [85].

Changing the delivery route was shown to bypass pre-existing immunity and maximize the efficacy of an Ad5 vaccine. The breadth of the immune response noted after nasal or oral immunization was superior to that after intramuscular administration of the vaccine [94]. Intranasal vaccination was observed as an effective vaccine delivery route in the presence of systemic or even mucosal pre-existing immunity to the Ad5 vector in guinea pigs [95], and use of hydrogel was found to be safe and effective as a delivery system for nasal immunization [96]. Of note, airway vaccination with an Ad5-based EBOV vaccine could efficiently bypass pre-existing immunity to Ad5 and induce protective immune responses in NHPs [97, 98]. Altogether, the available data have shown great potential for adenovirus-based vaccines to be licensed in the future.

In order to overcome the problem of pre-existing immunity, adenovirus vectors of different serotypes have been used. Ad26 and Ad35 are segregated genetically from Ad5 and have lower serum prevalence in humans, making them attractive alternative vaccine vectors [99]. Ad35 and Ad26 vaccines were shown to successfully induce potent cellular and humoral immune responses in mice after only a single vaccination. Furthermore, the Ad26 vaccine was advanced into clinical trials. The chimpanzee AdC7 is also a potential platform to which humans would have a low risk of pre-existing immunity, and it was demonstrated to provide full protection against EBOV in a guinea pig model [92]. Another chimeric vector was constructed using simian adenovirus 21/22 expressing EBOV GP (Ad C5/C1-ZGP), and a single administration of this vector protected mice against a lethal challenge of EBOV [100]. A replication-defective recombinant chimpanzee Ad3-vectored EBOV vaccine (ChAd3-EBOV) encoding GP from the Zaire and Sudan species was effective in the NHP model and was rapidly advanced into a Phase 1 clinical trial. Two doses at 2×10^{10} and 2×10^{11} particle units were deemed safe and effective in inducing cellular and humoral immunity, with the higher dose being more immunogenic [101]. When ChAd3-EBOV was boosted with MVA-BN filo, durable protection was found against lethal EBOV challenge. ChAd3-EBOV was further tested in another clinical trial at 1×10^{10} , 2.5×10^{10} and 5×10^{10} viral particles (20 participants per group), and preliminary data showed that the vaccine was immunogenic at the doses tested [102]. A Phase 2 clinical study of ChAd3-EBOV has been conducted, although the results are not yet available; recently, it was placed into a Phase 3 clinical study [103].

Vesicular stomatitis virus (VSV)-based vaccines

VSV is a prototypic member of the Rhabdoviridae family [104], and Rose et al. pioneered its use as a vaccine vector [105]. A recombinant VSV (rVSV) containing the EBOV GP gene in place of the VSV G-protein gene was used in a study aimed towards identifying amino acids recognized by neutralizing antibodies [106-108]. The rVSV vaccine was demonstrated to be

highly potent and safe. Moreover, protection by mucosal delivery was as effective as that by systemic injection [109]. The rVSV vaccine also has demonstrated the potential to confer cross-protection between ebolavirus species [110] and was the first replication-competent ebolavirus vaccine to show protection in NHPs [111, 112]. The protection afforded by the rVSV vaccine was durable [113] and effective against aerosol exposure to EBOV in NHPs [114].

As the effects of the rVSV vaccine were found to be particularly robust, it may serve not only in a prophylactic setting but also confer post-exposure protection. The rVSV vaccine expressing SUDV GP was shown to provide complete post-exposure protection when given to four rhesus macaques shortly after exposure to SUDV [115]. A bivalent vaccine expressing the EBOV and Andes virus glycoproteins were also evaluated, but it showed slightly reduced post-exposure efficacy due to its restricted replication in lymphoid organs [116].

Since VSV-based vaccines are replication competent, questions have been raised regarding their suitability for use in humans. To address these concerns, the safety profile of VSV-GP was evaluated in NHPs infected with simian-human immunodeficiency virus (SHIV). None of the six macaques in the study showed evidence of illness associated with the rVSV vaccine, suggesting that it would be safe for use in immune-compromised individuals [117]. Furthermore, Geisbert et al. confirmed the lack of neurovirulence of rVSV in NHPs [118]. Interestingly, the rVSV vaccine was once administered to a researcher exposed to EBOV, who did not become ill and remained healthy [119]. While this observation supports the safety of the rVSV vaccine in humans, additional work is needed to promote its licensure and approval for clinical use. An attenuated, replication-competent, recombinant VSV-based vaccine (rVSV-EBOV) was constructed without the VSV G gene and tested in clinical trials. This vaccine was given at the dose of 3×10^6 plaque-forming units (PFU) or 2×10^7 PFU to the volunteers. The most common adverse events were injection-site pain, myalgia and fatigue, but they were limited in duration. Meanwhile, transient VSV viremia was noted in all vaccine recipients, but no events resulted in withdrawal from the study. Anti-Ebola immune responses were identified in all the volunteers [120]. Another trial was conducted with 158 healthy adults in Europe and Africa, and that vaccine was given at several doses ranging from 3×10^5 to 5×10^7 PFU to the volunteers. Though neutralizing antibodies were induced, side effects such as transient vaccine viremia, fever and arthritis were observed in some individuals, warranting further evaluations for safety and selection of the optimal vaccination dose [121]. When reducing the dose of rVSV-EBOV to 3×10^5 PFU, early tolerability was improved, but lowered antibody responses were found; moreover, the low dose did not prevent vaccine-induced arthritis and dermatitis [122]. Encouragingly, rVSV-EBOV was reportedly effective in a Phase 3 clinical trial, with immediate vaccination at the dose of 2×10^7 PFU providing complete protection. Although side effects were not avoided, rVSV-EBOV may be considered highly efficacious and safe as a vaccine for preventing EVD [123].

Conclusions

Ebolavirus vaccines have been investigated for more than 30 years. Earlier in that period, progress was slow due to incomplete understanding of the virus and the lack of commercial interest for vaccine development. With the current knowledge of ebolaviruses and concern of their use in bioterrorism, vaccine development against EVD has become a priority for many nations. Especially after the finding of the first patient in the United States in September 2014, the need for an Ebola vaccine has attracted the attention of the world. Significant progress has been made recently in the development of ebolavirus vaccines. Although none has been licensed, several vaccine candidates have been evaluated and demonstrated efficacy in the NHP model. Ad5-based vaccines and DNA vaccines have successfully entered Phase I clinical trials, and a rVSV vaccine was used to protect one researcher with lab exposure to EBOV [106].

Currently, Ad26-EBOV, ChAd3-EBOV, rVSV-EBOV and MVA-BN filoviruses are the most promising candidates for ebolavirus infection. Ad26-EBOV and MVA-BN filoviruses were entered into a Phase 3 clinical trial [54]. ChAd3-EBOV successfully passed through early clinical trials and entered into a Phase 3 study [102,103], while rVSV-EBOV has already been validated to be effective in a Phase 3 clinical trial [123]. Challenges remain to prolong the protective effects of the vaccines, reduce adverse reactions and determine their mechanisms of protection.

Peptide vaccines also are another promising platform due to their relative safety, rapid induction of effective immune responses and available methods for high-level production. Another research direction should be to explore the combination of ebolavirus vaccines with effective adjuvants. Cytokine adjuvants may enhance the immunogenicity of ebolavirus vaccines and optimize the immune responses. Since ebolaviruses can easily be transmitted from human to human through body fluids, classical approaches of disease prevention, such as education and proper patient isolation, are extremely useful in prohibiting the spread of EVD. The protection of vaccines should cover high-risk groups in epidemic areas, healthcare workers, NHPs in Africa, as well as accidental laboratory exposures. More funding and research effort should be placed into the development of vaccines as well as identification of the reservoir(s) and potential interim hosts for ebolaviruses.

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Disclosure Statement

No conflict of interests.

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