Published in final edited form as:

Rev Med Virol. 2010 November ; 20(6): 344–357. doi:10.1002/rmv.661.

Prospects for immunisation against Marburg and Ebola viruses

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SUMMARY

For more than 30 years the filoviruses, Marburg virus and Ebola virus, have been associated with periodic outbreaks of hemorrhagic fever that produce severe and often fatal disease. The filoviruses are endemic primarily in resource-poor regions in Central Africa and are also potential agents of bioterrorism. Although no vaccines or antiviral drugs for Marburg or Ebola are currently available, remarkable progress has been made over the last decade in developing candidate preventive vaccines against filoviruses in nonhuman primate models. Due to the generally remote locations of filovirus outbreaks, a single-injection vaccine is desirable. Among the prospective vaccines that have shown efficacy in nonhuman primate models of filoviral hemorrhagic fever, two candidates, one based on a replication-defective adenovirus serotype 5 and the other on a recombinant VSV (rVSV), were shown to provide complete protection to nonhuman primates when administered as a single injection. The rVSV-based vaccine has also shown utility when administered for postexposure prophylaxis against filovirus infections. A VSV-based Ebola vaccine was recently used to manage a potential laboratory exposure.

INTRODUCTION

Marburg virus (MARV) and Ebola virus (EBOV), the causative agents of Marburg and Ebola hemorrhagic fever (HF), respectively, represent the two genera that comprise the family *Filoviridae* [1,2]. Filoviruses are filamentous, enveloped, non-segmented, negative sense RNA viruses with genomes of approximately 19 kb. Each virus encodes seven structural gene products: nucleoprotein (NP), virion protein (VP)35, VP40, glycoprotein (GP), VP30, VP24 and the polymerase (L). In addition, EBOV expresses at least one non-structural soluble GP (sGP) encoded by the GP gene [1,2]. The GP, with variable contribution from the NP, appears to be the key immunogenic protein in vaccine protection.

The *Marburgvirus* genus contains a single species, *Lake Victoria marburgvirus*, while the *Ebolavirus* genus is comprised of four distinct species: (1) *Sudan ebolavirus* (SEBOV), (2) *Zaire ebolavirus* (ZEBOV), (3) *Cote d'Ivoire ebolavirus* (also known and here referred to as *Ivory Coast ebolavirus* (ICEBOV)) and (4) *Reston ebolavirus* (REBOV) [1]. A putative fifth species, *Bundibugyo ebolavirus* (BEBOV), was associated with an outbreak in Uganda in 2007 [3]. MARV, ZEBOV, SEBOV and BEBOV are important human pathogens with case fatality rates frequently ranging up to 90% for MARV and ZEBOV, and around 55% for SEBOV (reviewed in Ref. [2]). Based on a single outbreak, the newly discovered BEBOV appears to be less pathogenic with a case fatality rate of about 25% [3]. ICEBOV caused deaths in chimpanzees and a severe nonlethal human infection in a single case in the Republic of Cote d'Ivoire in 1994 [4]. REBOV is lethal for macaques but has not yet been associated with disease in humans [2]. An outbreak of REBOV has recently been discovered in pigs in the Philippines; however, it is unclear whether the disease observed in the pigs was caused by REBOV or other agents shown to be co-infecting the animals, especially porcine reproductive and respiratory syndrome virus [5].

While there are no vaccines approved by the US Food and Drug Administration (FDA) or postexposure treatments available for preventing or managing EBOV or MARV infections, there are at least five different vaccine systems that have shown promise in completely protecting nonhuman primates (NHPs) against EBOV and four of these have also been shown to protect macaques against MARV infection [6–19]. Although Marburg and Ebola HF are rare diseases, a preventive vaccine could be considered important for several populations: (1) The general population during filovirus outbreaks in endemic areas in sub-Saharan Africa or related to imported cases of filovirus infection in humans or NHPs; (2) healthcare workers and family members involved in patient care and management in these same areas; (3) personnel involved in outbreak response missions; (4) laboratory workers conducting research on filoviruses and (5) military and other service personnel susceptible to use of filoviruses as a bioweapon.

The desirable characteristics of the filovirus vaccines required by these diverse groups may vary. While laboratory and healthcare workers and some military personnel in stable settings with defined risk may be candidates for a multi-dose vaccine, outbreak settings will require protection to be rapidly conferred with a single administration. Similarly, in the case of a deliberate release of these agents there would be little time for deployment of a vaccine that requires multiple injections. The required durability of protection may also vary. Military personnel, laboratory or healthcare workers rotating through high risk situations for fixed periods may make due with a limited duration of protection, perhaps as short as a year, while long-term protective efficacy is desirable for those with more chronic exposure. The ideal vaccine meeting all needs would confer long-term protection with little or no filovirus viremia against ZEBOV, SEBOV, BEBOV and the diverse strains of MARV with a single administration.

ANIMAL MODELS

Rodents including guinea pigs, mice and hamsters have been used as animal models of filovirus HF [20–25]. Because filovirus isolates derived from humans or NHPs do not typically produce severe disease in rodents upon initial exposure, serial adaptation is required to produce a uniformly lethal infection. Mice and guinea pigs have served well as early screens for evaluating antiviral drugs and candidate vaccines, and genetically engineered mice clearly have utility for dissecting out specific host–pathogen interactions. However, the disease pathogenesis seen in rodent models is far less faithful in portraying the human condition than disease observed in NHPs [26,27]. As examples, coagulation disorders that are hallmark features of disease in filovirus-infected humans and NHPs are

not present in filovirus-infected mice or guinea pigs [26,27]. Also, while the bystander death of large numbers of uninfected lymphocytes due to apoptosis has been reported in filovirus-infected humans [28], NHPs [29] and mice [30], the process and morphology of lymphocyte apoptosis is different among primates and mice [30]. Because data derived from studies using rodents may not correlate with human disease, and because rodent models have not accurately predicted results in the more robust NHP models [31–34] this review focuses on vaccine studies performed in NHPs.

HISTORICAL PERSPECTIVE

The effort to develop filovirus vaccines began after the initial identification of MARV in 1967 and EBOV in 1976. Early attempts were based on classical approaches of using inactivated whole virion preparations as vaccines. Two studies using formalin- or gamma-inactivated whole MARV virions in rhesus macaques or African green monkeys were able to demonstrate protection of only about half of the animals against homologous MARV challenge [35–37]. Evaluation of humoral and cellular immune responses in one of these studies showed that there was considerable variability in the immune responses among the cohort of six macaques used, with no correlation between the measured immune response and survival [37]. The authors concluded that protective immunity in this case was largely determined by indices of nonspecific immunity.

Results from EBOV studies were also inconsistent. Mikhailov *et al.* [38] were the first to demonstrate significant protection of NHPs against lethal filoviral challenge, protecting four of five hamadryas baboons (*Papio hamadryas*) with a formalin-inactivated purified whole virion ZEBOV vaccine. In another study, only one of four macaques vaccinated with a gamma-irradiated ZEBOV whole virion preparation survived lethal challenge [27]. Liposomes containing lipid A were also evaluated as a delivery system for inactivated ZEBOV antigens in hope of enhancing antibody and cellular immune responses [39]; however, this strategy failed to protect any cynomolgus monkeys from lethal ZEBOV infection [27]. Overall, vaccine candidates based on inactivated virus preparations have not shown promising efficacy in NHP models. Due to the potential remaining risk of improper inactivation, these types of vaccines are unlikely to be licensed for a BSL-4 pathogen.

RECOMBINANT GENE-BASED VACCINES

Recent efforts to develop vaccines for filovirus HFs have concentrated on the use of various recombinant vectors for the delivery of genes expressing filovirus proteins to induce protective immunity (Tables 1 and 2). Delivery systems used to express filovirus proteins for these purposes include vaccinia viruses, Venezuelan equine encephalitis virus (VEEV) replicons, DNA-based vaccines, adenoviruses, vesicular stomatitis virus (VSV), human parainfluenza virus type 3 (HPIV3) and virus-like particles (VLPs).

Recombinant vaccinia viruses

Vaccinia virus has been the most intensively studied live recombinant vaccine vector (reviewed in Ref. [40]). In most cases, recombinant vaccinia viruses are constructed by exploiting homologous DNA recombination in vaccinia virus-infected cells where the foreign viral gene is inserted into a shuttle vector that is flanked by poxvirus sequences. While recombinant vaccinia viruses have shown utility as vaccine vectors against a number of infectious agents, a few studies have evaluated this platform for filoviruses. One study showed that recombinant vaccinia viruses expressing the ZEBOV GP were unable to prolong survival or protect cynomolgus monkeys from lethal ZEBOV infection [27].

Venezuelan equine encephalitis virus replicons

Alphaviruses have a broad host range and replicate in a variety of different vertebrate and invertebrate cells. The alphavirus genome consists of single-stranded, positive-sense RNA divided into two open reading frames, one encoding the nonstructural proteins responsible for transcription and replication, and a second typically encoding the structural proteins, which are responsible for encapsidation of viral RNA and final assembly into enveloped virions. Alphaviruses can be employed as vaccine vectors by cloning the gene for the protein of interest in place of the alphavirus structural genes. These RNA vectors, commonly called 'replicons', have the ability to replicate but are not packaged into virus particles in the absence of the alphaviral structural proteins. Therefore, alphavirus replicons are one-cycle vectors that are not capable of spreading from cell to cell. Expression vectors have been constructed from at least three different alphaviruses, including VEEV, Semliki Forest and Sindbis viruses [41–43].

Hevey *et al.* [6] tested VEEV replicons expressing MARV Musoke strain (MARV-Musoke) GP either alone or in combination with NP in cynomolgus macaques. The experiment consisted of three VEEV replicon injections spread 28 days apart followed by a high dose i.m. MARV challenge 35 days after the third immunisation. Animals vaccinated with either MARV-Musoke GP or GP and NP, were completely protected against a homologous MARV challenge. NP alone prevented death but not disease in two of three monkeys and all three animals became viremic. A similar strategy did not protect against challenge with the heterologous MARV Ravn strain (MARV-Ravn) [44], raising questions about the degree of cross-protection of candidate vaccines for the diverse strains of MARV. For EBOV, results in NHPs have not been as encouraging; vaccination of cynomolgus monkeys with VEEV replicons expressing either ZEBOV GP, NP or both GP and NP, failed to protect any animals from a lethal ZEBOV infection [27].

The VEEV replicon system currently faces a number of challenges. In addition to the aforementioned failure to protect against ZEBOV and inability to provide cross-protection between strains of MARV, even protection against homologous MARV required a series of three injections over 17 weeks. Protection might be improved by increasing the vaccine dose, but even with the dose used (10^7 pfu) monkeys developed VEEV-neutralising antibodies after two injections [45] raising doubts about the reusability of this system even if the problem of cross-protection can be solved.

DNA-based vaccines

Vaccines using plasmid DNA have proven to be one of the most promising applications in the field of gene therapy. Advantages of DNA vaccines include that they are amenable to rapid assembly and large-scale production, their potential to stimulate both humoral and cell-mediated immune responses, and the fact that they are reusable systems. However, while DNA vaccines have been remarkably effective in small animal models, their potency when transitioned to humans has most often been disappointing. Furthermore, because DNA vaccines are relatively new and represent a novel vaccine technology, certain safety issues, such as the potential for induction of autoimmune disease and integration into the host genome, must be examined carefully.

The DNA vaccine approach for filoviruses has met with mixed results in NHPs. Four of six cynomolgus macaques given a series of three vaccinations with MARV-Musoke GP DNA at 4-week intervals survived a high dose i.m. challenge (1000 pfu) of homologous MARV 3 weeks after the final vaccination [46]. Interestingly, although all six MARV vaccinated monkeys produced moderate serum IgM antibody titres, there was no apparent association between antibody level and survival, suggesting cell-mediated immunity was the primary

mediator of protection. The cellular immune response was not assessed in this study. Intramuscular injection of cynomolgus macaques with three doses of MARV-Angola strain (MARV-Angola) GP DNA followed by challenge with a high dose (1000 pfu) of homologous virus 28 days after the last injection resulted in survival of all four animals, but three of the four monkeys became clinically ill [47]. DNA vaccines have also been used in prime-boost strategies using a combination of different antigen delivery systems. Prime-boost strategies were tested for both MARV and EBOV in NHPs (see below under adenoviruses).

Adenoviruses

Adenoviruses are highly attractive vaccine vectors for gene therapy because of their high transduction efficiency, broad tropism and ability to induce both innate and adaptive immune responses in mammalian hosts. Despite setbacks, including the death of a patient in 1999 from adverse effects associated with the administration of adenovirus vectors [48], interest in their use has remained high, and efforts have focused on developing vectors that have low or no immunogenic toxicities.

Replication-defective adenoviruses, especially adenovirus serotype 5 (Ad5) are the most commonly used platform [49]. The common feature of all recombinant, replication-defective adenovirus vectors is deletion of the viral E1 region that is essential for the regulation of adenovirus transcription and viral replication. In addition, the E3 region, which is not essential for production of recombinant virus vector, is often deleted. The E4 region, which must be provided in *trans* for production of recombinant virus, can also be deleted to increase capacity for gene inserts and to reduce host responses *in vivo* [49].

In NHPs, the rAd5 platform has shown remarkable success for filovirus HFs [7,8,11,15,19]; notably, a single i.m. injection with a rAd5-based vaccine expressing MARV-Angola GP resulted in complete protection from death and illness of cynomolgus macaques after a high dose (1000 pfu) i.m. challenge with homologous MARV 28 days later [47]. A second set of four monkeys who received the rAd5 MARV-Angola GP vaccine after three injections of MARV-Angola GP DNA in a prime-boost strategy was also completely protected, but the failure of the DNA vaccine alone to protect against clinical illness (see above under DNA-based vaccines) suggests that rAd5 MARV was the key component [47].

The first success at completely protecting NHPs from ZEBOV HF was demonstrated by Sullivan *et al.* [7] using a prime-boost strategy; cynomolgus monkeys were vaccinated three times with DNA expressing GPs of ZEBOV, SEBOV and ICEBOV, and NP of ZEBOV followed 3 months later by a booster vaccination with a rAd5 vector expressing the ZEBOV GP. All four vaccinated animals survived challenge at week 32 of the vaccination regimen when exposed to a low dose (6 pfu) of ZEBOV. The results of this study showed that antibody and T memory helper cells were strongly associated with protection and also suggested that, while cell-mediated immunity was important, it was not an absolute requirement for protection [7]. The contribution of the DNA component of this regimen is not clear, since no studies have been reported on its efficacy when used alone, while the rAd5 component used alone is protective; as with MARV, a single injection of rAd5 expressing the ZEBOV GP resulted in complete protection from death and illness of cynomolgus macaques after a high dose (1000 pfu) i.m. challenge with homologous ZEBOV 28 days later [8].

While aerosol transmission is not thought to be a major route of filovirus infection in nature, the inhalation route is among the most likely portals of entry in the setting of a bioterrorist event. Recent studies showed that cynomolgus monkeys vaccinated once with a rAd5 vector expressing ZEBOV NP, ZEBOV GP and SEBOV GP were completely protected against an

aerosol ZEBOV challenge and partially protected against an aerosol SEBOV challenge [19]. Increasing the vaccination regimen to two injections over 99 days resulted in complete protection against a SEBOV aerosol challenge [19].

Recently, a two-injection filovirus vaccine was described that is based on a rAd5 vector expressing multiple antigens from five different filoviruses (ZEBOV NP, ZEBOV GP, SEBOV GP, MARV-Ci67 GP, MARV-Ravn GP, MARV-Musoke NP, MARV Musoke GP) [15]. In this study, two groups of cynomolgus monkeys were given an initial i.m. injection of this vaccination and then were revaccinated 63 days later. The first group was challenged with MARV-Musoke 42 days after the second vaccination and then back-challenged 72 days later with SEBOV. The second group was initially challenged with ZEBOV 43 days after the second vaccination and then back-challenged 69 days later with MARV-Ci67. All animals in these studies survived both challenges. A more recent study of the same vaccination strategy also showed protection against an initial SEBOV challenge [19].

The major drawback of rAd5-based vaccines is the high prevalence of pre-existing immunity to the adenoviruses that may substantially limit their immunogenicity and clinical utility. The prevalence of anti-adenovirus antibody is up to 60% in the general human population and up to 85% in Africa, where filovirus vaccines are most needed [50,51]. Related to this concern, Merck recently discontinued its HIV vaccine program based on rAd5 as it was reported that the vaccine appeared to increase the rate of HIV infection in individuals with prior immunity against the adenovirus vector used in the vaccine [52,53]. To date, attempts to improve adenovirus-based vaccines against filo-viruses by employing different adenovirus sero-types as vectors have been unsuccessful [54]. Specifically, vaccination of cynomolgus monkeys with adenovirus serotype 35 expressing the ZEBOV GP failed to completely protect animals against a lethal ZEBOV challenge. Likewise, vaccination of cynomolgus macaques with either adenovirus serotype 26 or a modified adenovirus in which only the seven short hexon hypervariable regions of Ad5 were exchanged from human adenovirus serotype 48 (each expressing the ZEBOV GP) failed to protect animals against a lethal ZEBOV challenge. Thus, to date, Ad5 is the only human adenovirus serotype capable of inducing a protective response against EBOV [54]. Unfortunately, when macaques are pre-immunised against Ad5, vaccinated with the rAd5 vaccine expressing the ZEBOV GP, and then challenged with ZEBOV they are not protected against disease or death [54].

Vesicular stomatitis virus

In the last few years, Rose and colleagues have pioneered the use of VSV, the prototypic member of the *Rhabdoviridae* family, as an expression and vaccine vector [55–57]. VSV possesses numerous favourable characteristics as a vaccine expression vector, including ease of growth to high titre ($>10^9$ pfu/ml) *in vitro*, propagation in almost all mammalian cells, induction of strong humoral as well as cellular responses *in vivo*, and the capacity to elicit both mucosal and systemic immunity [57–60]. Furthermore, human infection with VSV is rare and not typically associated with serious disease, although VSV-associated encephalitis has been reported, so pre-existing immunity should not pose a problem [61–63].

A recombinant VSV (rVSV)-based system has proven to be the most successful vaccine platform for MARV to date, and has been proven equally effective against EBOV. A single i.m. vaccination of cynomolgus monkeys with a rVSV MARV-Musoke GP vector elicited complete protection against a high dose (1000 pfu) i.m. challenge of homologous MARV given 28 days later [9]. The animals were also protected upon rechallenge 113 days later. Furthermore, the same vaccine proved protection against the most genetically disparate MARV strain, Ravn, and what appears to be the most virulent strain, Angola, suggesting that it may confer cross-protection against all the diverse strains of MARV [10]. Recent

studies showed that a single vaccination of cynomolgus monkeys with rVSV MARV-Musoke GP completely protected animals against a homologous aerosol challenge of MARV given 28 days later [14].

For EBOV, a single i.m. vaccination of cynomolgus monkeys with a rVSV vector expressing only ZEBOV GP elicited complete protection against a high dose (1000 pfu) i.m. challenge of homologous ZEBOV given 28 days later [9]. However, there was no cross-protection, as subsequent back-challenge of the ZEBOV survivors with SEBOV resulted in fatal disease [9]. Similar to MARV, a single i.m. vaccination of cynomolgus monkeys with rVSV ZEBOV GP completely protected animals against a homologous aerosol challenge of ZEBOV given 28 days later [14]. Furthermore, protection can be conferred by these vaccines via various delivery routes; immunisation of cynomolgus monkeys with the rVSV ZEBOV GP vector by either the intranasal or oral route resulted in complete protection of all animals against a high dose (1000 pfu) i.m. homologous ZEBOV challenge [18].

Due to possible overlapping endemicities of the filoviruses in Africa, as well as the threat of bioterrorism, it would be ideal to have a single-injection vaccine that could protect against the various species and strains of EBOV and MARV. In a recent study, cynomolgus monkeys were vaccinated with a multivalent vaccine consisting of equal parts of the rVSV GP vaccine vector for MARV, EBOV and SEBOV [17]. Four weeks later groups of the animals were challenged with either MARV, ZEBOV, SEBOV or ICEBOV. Importantly, none of the vaccinated macaques succumbed to a filovirus challenge, showing great promise with this single-injection multivalent platform.

In addition to its utility as a preventive vaccine, the rVSV vaccine platform has also been used as postexposure prophylaxis for filovirus infections (Table 3). Administration of rVSV MARV Musoke GP to rhesus monkeys shortly after a homologous high-dose MARV challenge resulted in complete protection of all animals from clinical illness and death [64]. Subsequent studies demonstrated that the rVSV GP vectors for ZEBOV and SEBOV protected 50 and 100%, respectively, of rhesus macaques when administered as postexposure prophylaxis after high-dose homologous virus challenge [65,66]. The vaccine was administered in these studies 20–30 min after filovirus challenge. A major question is how long after virus exposure can the rVSV vaccine be effective for an acute infection with an incubation period averaging 4–10 days? In a recent study, treatment of rhesus monkeys with rVSV MARV-Musoke GP 24 h after homologous MARV challenge resulted in protection of five of six monkeys while, remarkably, two of six animals were protected when the vaccine was administered 48 h after infection [67].

The main concern with all replication-competent vaccines, including the rVSV platform, is their safety, especially in immunocompromised persons. However, initial results of various rVSV vectors in NHPs are promising; no toxicity was seen in rhesus macaques following intranasal inoculation with wild type VSV, rVSV and two rVSV-HIV vectors, although neurovirulence was noted in one of four animals after direct intrathalamic inoculation of rVSV [68]. To date, no toxicity has been seen in over 80 NHPs given rVSV MARV or EBOV [9,10,14,17,18,69]. Furthermore, no significant vaccine vector shedding has been seen in these experiments despite challenge doses of up to 10^7 pfu [9,10,14,17,18,69] which suggests, along with the natural low transmissibility of VSV [70,71], that spread to persons outside the vaccine target population is unlikely.

To specifically address its safety, the rVSV ZEBOV GP vaccine was evaluated in two animal models for the immunocompromised state, NODSCID mice [72] and SHIV-infected rhesus monkeys [69]. No evidence of overt illness was noted in any of the animals. In addition, the rVSV ZEBOV GP vector was recently used to manage a laboratory worker after

a recent laboratory accident [73]. The vector was administered around 40 h after potential ZEBOV exposure. The patient developed fever, headache and myalgia 12 h after injection which were readily controlled with antipyretics and analgesics. No other adverse effects were reported. Because it is not certain that infection actually occurred, efficacy of the vaccine in this case could not be evaluated. Lastly, to further ensure safety, the VSV GP, to which the pathogenicity of wild-type VSV may be attributable [74], has been deleted in the rVSV filovirus vectors. Regarding possible vaccine virus mutation to more virulent variants, some comfort can be taken from noting the case of the live recombinant vaccinia vaccine for rabies that has been under field investigation in wild animals in the United States, Canada and Europe since the 1980s and 1990s [75] with no evidence of evolution to more pathogenic forms.

Human parainfluenza virus type 3

HPIV3 is a member of the family *Paramyxoviridae* and a common paediatric respiratory pathogen. Live-attenuated vectors based on HPIV3 are actively being investigated as vaccines for HPIV3 and other paediatric pathogens [76,77]. Bukreyev *et al.* [12] recently developed HPIV3 vectors expressing the ZEBOV GP and the ZEBOV GP and NP. A combination of intranasal and intratracheal vaccination of cynomolgus monkeys with the ZEBOV GP afforded the best protection against a high dose (1000 pfu) intraperitoneal challenge of homologous ZEBOV 28 days after immunisation [12]. In this study, six of seven HPIV3 ZEBOV GP-vaccinated animals survived challenge and four of seven animals were protected against clinical illness. Two doses of the HPIV3 ZEBOV GP vaccine over a 67-day period appeared to improve efficacy, completely protecting three of three cynomolgus monkeys against clinical illness and death.

The replication-competent nature of the HPIV3 system brings similar safety concerns as the rVSV platform. In addition, nearly all adult humans have pre-existing immunity to this common childhood pathogen, presenting a potential challenge in immune response to the vaccine vector analogous to rAd5. However, Bukreyev *et al.* [78] recently demonstrated that re-infection of NHPs with HPIV3 expressing ZEBOV GP is achievable and results in an appropriate immune response, indicating that vaccination might be feasible despite pre-existing immunity. Unfortunately, this study did not provide data on protective efficacy.

Virus-like particles

VLPs are a specific type of subunit vaccine that structurally mimic authentic virions but do not contain infectious genetic material, and are thus theoretically safer than most live virus preparations. Another advantage of VLPs as immunogens is that they are not subject to problems associated with pre-existing anti-vector immunity. VLPs have been constructed for more than 30 different viruses, including EBOV and MARV [79–81]. Formation of EBOV and MARV VLPs requires, at a minimum, expression of the filoviral matrix protein, VP40. Inclusion of other proteins such as NP and GP can enhance the budding and release of VP40 VLPs [80].

Recently, the efficacy of a MARV vaccine consisting of VLPs expressing MARV-Musoke GP, NP and VP40 was assessed in cynomolgus monkeys; animals received three i.m. injections of the MARV VLPs with QS-21 adjuvant at 42-day intervals [16]. Cohorts of three animals each were challenged i.m. 4 weeks after the final vaccination with 1000 pfu of MARV-Musoke, MARV-Ci67 or MARV-Ravn. Animals challenged with homologous MARV-Musoke or with MARV-Ci67 were completely protected from death and clinical illness while animals challenged with MARV-Ravn were completely protected from death but not clinical illness. This is not surprising as MARVRavn is the most genetically distant strain of MARV. For EBOV, a ZEBOV vaccine consisting of VLPs expressing ZEBOV GP,

NP and VP40 was evaluated in cynomolgus monkeys [13]; animals received three i.m. injections of the ZEBOV VLPs with RIBI adjuvant at 42-day intervals. All of the macaques were completely protected against a high dose (1000 pfu) i.m. homologous ZEBOV challenge 4 weeks after the final vaccination.

There are considerable logistical hurdles to be overcome if the potential of VLPs as a MARV or EBOV vaccine is to be realised. The current laboratory process employing eukaryotic cells does not readily produce a compositionally and structurally consistent product when scaled up [82]. Alternative processes, such as insect cell-derived VLPs [83], plant derived VLPs [84] or chemical self-assembly are at early stages of development and must be proven. Insect cell-derived VLPs are being employed by GlaxoSmithKline for their VLP-based human papilloma virus (HPV) vaccine [85] that was recently approved for human use. The chemical self-assembly process is currently being employed by Merck for their VLP-based HPV vaccine that was shown to be immunogenic and highly protective in human clinical trials [86] and was recently licensed for human use. These vaccines may pave the way for other VLP-based vaccines. However, the HPV vaccines only protect against 2 of the 15 oncogenic genital HPV types (reviewed in Ref. [87]) suggesting that it may prove challenging to make an analogous VLP-based vaccine protecting against the five different species of EBOV and several genetically distinct strains of MARV. Another major challenge for VLP-based filovirus vaccines is the number of vaccinations (three times) and length of time (~154 days) required to achieve a protective immune response.

CONCLUSION

Significant progress has been made over the last several years in developing potential vaccines for filovirus HF. At least four systems have shown the ability to protect NHPs against lethal MARV and EBOV challenges and one of these, rVSV, was even shown to be effective as postexposure prophylaxis. Results of studies using all systems show that a multivalent filovirus vaccine will require at least three components, MARV GP, ZEBOV GP and SEBOV GP. Further studies will need to be performed to determine whether ZEBOV GP or SEBOV GP can protect against BEBOV HF or whether BEBOV antigens will be required in a panfilovirus vaccine.

A thorough understanding of the pathogenesis of filoviruses in relevant animal models is essential not only for further evaluation of the efficacy of existing vaccine candidates, but also in light of the so called 'animal rule' enacted by the U.S. FDA in 2002 (reviewed in Ref. [88]), which establishes requirements for the evidence needed to demonstrate effectiveness of new drugs and biological products when human efficacy studies are not ethical or feasible, as would likely be the case for filoviral HF. Under the rule, a product can be licensed based on evidence of effectiveness derived from studies in well-characterised animal models, in addition to the usual demonstration of biological activity and safety in humans. Thus, the validation of NHPs as accurate and reliable models of human filoviral HF will be critical to the final evaluation and testing of candidate vaccines. Ultimately, no vaccine for MARV or EBOV infection will be approved for human use until it can protect NHPs from clinical illness and viremia.

The characterisation of filovirus pathogenesis in laboratory animal models will need to be corroborated by findings in humans. To do this, more effort will need to be directed toward the application of modern immunological and molecular techniques to the study of human filovirus infection during sporadic outbreaks in Africa [89]. In particular, a deeper understanding of the correlates of immunity in both humans and animal models of filovirus HF will be essential.

Lastly, further down the road are questions about economic incentives for pharmaceutical companies to produce a MARV or EBOV vaccine should FDA approval eventually be granted. Filovirus HF is extremely rare and filoviruses are endemic in what are generally the world's poorest countries, so economic incentives will have to come from elsewhere. Industrialised countries' concerns about protecting the military and others considered susceptible to use of filoviruses as bioweapons are the most likely driving force. Responsibility will then fall on the international community to ensure that these vaccines make their way to persons in need in endemic areas for the filoviruses.

Abbreviations used

Ad5	adenovirus serotype 5
EBOV	Ebola virus
FDA	Food and Drug Administration
GP	glycoprotein
HF	hemorrhagic fever
HPIV3	human parainfluenza virus type 3
HPV	human papillomavirus
ICEBOV	Ivory Coast ebolavirus
L	polymerase
MARV	Marburg virus
MARV-Angola	MARV-Angola strain
MARV-Musoke	MARV-Musoke strain
MARV Ravn	MARV-Ravn strain
NHP	nonhuman primates
NP	nucleoprotein
REBOV	Reston ebolavirus
rVSV	recombinant VSV
sGP	soluble GP
SEBOV	Sudan ebola-virus
VEEV	Venezuelan equine encephalitis virus
VLPs	virus-like particles
VP	virion protein
ZEBOV	Zaire ebolavirus

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Table 1

Approaches to preventive Marburg virus vaccines in nonhuman primates (NHP)

System	Gene Product (Species)	Vaccine Dose	No. of Doses	NHP Species	Challenge Strain	Survivors / Total	Viremic/Total	Illness/Total	Ref
VEEV replicon	GP (Musoke)	10 ⁶ 7	3	Cynomolgus	Musoke *	3/3	0/3	0/3	[6]
VEEV replicon	GP (Musoke) + NP (Musoke)	10 ⁶ 7	3	Cynomolgus	Musoke *	3/3	0/3	0/3	[6]
VEEV replicon	NP (Musoke)	10 ⁶ 7	3	Cynomolgus	Musoke *	2/3	3/3	3/3	[6]
VEEV replicon	GP (Musoke)	10 ⁶ 7	3	Cynomolgus	Ravn *	0/3	NR	3/3	[44]
VEEV replicon	GP (Musoke) + NP (Musoke)	10 ⁶ 7	3	Cynomolgus	Ravn *	0/3	NR	3/3	[44]
DNA plasmid	GP (Musoke)	20 µg	3	Cynomolgus	Musoke *	4/6	2/6	6/6	[46]
DNA plasmid	GP (Angola)	4 mg	4	Cynomolgus	Angola *	4/4	0/4	3/4	[47]
DNA prime + Ad5 boost	GP (Angola) for both	4 mg	3	Cynomolgus	Angola *	4/4	0/4	2/4	[47]
		10 ⁶ 11	1						
Ad5	GP (Angola)	10 ⁶ 11	1	Cynomolgus	Angola *	4/4	0/4	0/4	[47]
Ad5	GP (Z) + NP (Z) + GP (S) + GP (Ci67) + GP (Ravn)	10 ⁶ 10	2	Cynomolgus	Musoke *	5/5	NR	1/5	[15]
VSV	GP (Musoke)	10 ⁶ 7	1	Cynomolgus	Musoke *	4/4	0/4	0/4	[9]
VSV	GP (Musoke)	10 ⁶ 7	1	Cynomolgus	Musoke *	1/1	0/1	0/1	[10]
VSV	GP (Musoke)	10 ⁶ 7	1	Cynomolgus	Ravn *	3/3	0/3	0/3	[10]
VSV	GP (Musoke)	10 ⁶ 7	1	Cynomolgus	Angola *	3/3	0/3	0/3	[10]
VSV	GP (Musoke)	10 ⁶ 7	1	Cynomolgus	Musoke **	4/4	0/3	0/3	[14]
VSV	GP (Z) + GP (S) + GP (M-Musoke)	10 ⁶ 7	1	Cynomolgus	Musoke *	3/3	0/3	0/3	[17]
VLP	GP (Musoke) + NP (Musoke) + VP40 (Musoke)	1 mg	3	Cynomolgus	Musoke *	3/3	0/3	0/3	[16]
VLP	GP (Musoke) + NP (Musoke) + VP40 (Musoke)	1 mg	3	Cynomolgus	Ci67 *	3/3	0/3	0/3	[16]
VLP	GP (Musoke) + NP (Musoke) + VP40 (Musoke)	1 mg	3	Cynomolgus	Ravn *	3/3	0/3	1/3	[16]

Abbreviations: Ad5-adenovirus serotype 5, GP-glycoprotein, NP-nucleoprotein, NR-not reported, S-Sudan ebolavirus, VEEV-Venezuelan equine encephalitis virus, VLP-Virus-like particle, VP-viral protein, VSV-Vesicular stomatitis virus, Z-Zaire ebolavirus.

* Intramuscular.
** Aerosol.

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Table 2

Approaches to preventive Ebola virus vaccines in nonhuman primates (NHP)

System	Gene Product (Species)	Vaccine Dose	No. of Doses	NHP Species	Challenge Species	Survivors/Total	Viremic/Total	Illness/Total	Ref
Vaccinia	GP (Z)	10 ⁶ 7	3	Cynomolgus	Zaire *	0/3	3/3	3/3	[27]
VEEV replicon	GP (Z)	10 ⁶ 7	3	Cynomolgus	Zaire *	0/3	3/3	3/3	[27]
VEEV replicon	NP (Z)	10 ⁶ 7	3	Cynomolgus	Zaire *	0/3	3/3	3/3	[27]
VEEV replicon	GP (Z) + NP (Z)	10 ⁶ 7	3	Cynomolgus	Zaire *	0/3	3/3	3/3	[27]
DNA prime	DNA: GP (Z) + GP (S) + GP (IC) + NP (Z); Ad5: GP(Z)	4 mg	3	Cynomolgus	Zaire *	4/4	1/4	0/4	[7]
+ Ad5 boost									
Ad5	GP (Z) + NP (Z)	10 ⁶ 10	1						
Ad5	GP (Z) + NP (Z)	10 ⁶ 12	1	Cynomolgus	Zaire *	4/4	0/4	0/4	[8]
Ad5	GP (Z) + NP (Z)	10 ⁶ 12	2	Cynomolgus	Zaire *	4/4	0/4	0/4	[8]
Ad5	GP (Z) + NP (Z)	10 ⁶ 12	1	Cynomolgus	Zaire *	4/4	0/4	0/4	[11]
Ad5	GP (Z) + NP (Z)	10 ⁶ 11	1	Cynomolgus	Zaire *	3/3	0/3	0/3	[11]
Ad5	GP (Z) + NP (Z)	10 ⁶ 10	1	Cynomolgus	Zaire *	6/6	0/6	0/6	[11]
Ad5	GP (Z) + NP (Z)	10 ⁶ 9	1	Cynomolgus	Zaire *	0/3	3/3	3/3	[11]
Ad5	GPATM (Z) + NP (Z)	10 ⁶ 12	1	Cynomolgus	Zaire *	2/3	1/3	1/3	[11]
Ad5	GPATM (Z) + NP (Z)	10 ⁶ 11	1	Cynomolgus	Zaire *	1/3	2/3	2/3	[11]
Ad5	GP (Z) E71D + GP (S) E71D + NP (Z)	10 ⁶ 10	1	Cynomolgus	Zaire *	1/3	2/3	2/3	[11]
Ad5	GP (Z) E71D + GP (S) E71D	10 ⁶ 10	1	Cynomolgus	Zaire *	3/3	0/3	0/3	[11]
Ad5	GP (Z) E71D + NP (Z)	10 ⁶ 10	1	Cynomolgus	Zaire *	2/3	1/3	1/3	[11]
Ad5	GP (Z)+NP (Z) + GP (S) + GP (M-Ci67) + GP (M-Ravn)	10 ⁶ 10	2	Cynomolgus	Zaire *	5/5	NR	0/5	[15,19]
Ad5	GP (Z)+NP (Z) + GP (S) + GP (M-Ci67) + GP (M-Ravn)	10 ⁶ 10	2	Cynomolgus	Sudan *	5/5	NR	0/5	[19]
Ad5	GP (Z) + NP (Z) + GP (S)	10 ⁶ 10	1	Cynomolgus	Zaire **	3/3	NR	0/3	[19]
Ad5	GP (Z) + NP (Z) + GP (S)	10 ⁶ 10	1	Cynomolgus	Sudan **	2/3	NR	2/3	[19]

System	Gene Product (Species)	Vaccine Dose	No. of Doses	NHP Species	Challenge Species	Survivors/Total	Viremic/Total	Illness/Total	Ref
Ad5	GP (Z) + NP (Z) + GP (S)	10 ⁶ 10	2	Cynomolgus	Sudan ^{**}	3/3	0/3	1/3	[19]
VSV	GP (Z)	10 ⁶ 7	1	Cynomolgus	Zaire [*]	4/4	0/4	0/4	[9]
VSV	GP (Z)	10 ⁶ 7	1	Cynomolgus	Zaire ^{**}	3/3	0/3	0/3	[14]
VSV	GP (Z) + GP (S) + GP (M-Musoke)	10 ⁶ 7	1	Cynomolgus	Zaire [*]	3/3	0/3	0/3	[17]
VSV	GP (Z) + GP (S) + GP (M-Musoke)	10 ⁶ 7	1	Cynomolgus	Sudan [*]	2/2	0/2	0/2	[17]
VSV	GP (Z) + GP (S) + GP (M-Musoke)	10 ⁶ 7	1	Cynomolgus	Ivory Coast [*]	3/3	0/3	0/3	[17]
VSV	GP (Z)	10 ⁶ 7	1	Cynomolgus	Sudan [*]	0/1	1/1	1/1	[17]
VSV	GP (Z) + GP (S) + GP (M-Musoke)	10 ⁶ 7	2	Rhesus	Sudan [*]	3/3	0/3	0/3	[17]
VSV	GP (Z) - Oral	10 ⁶ 7	1	Cynomolgus	Zaire [*]	4/4	NR	0/4	[18]
VSV	GP (Z) - IN	10 ⁶ 7	1	Cynomolgus	Zaire [*]	4/4	NR	0/4	[18]
HPV3	GP (Z)	10 ⁶ 6	1	Rhesus	Zaire ^{***}	4/4	0/4	1/4	[12]
HPV3	GP (Z)	10 ⁶ 7	1	Rhesus	Zaire ^{***}	2/3	2/3	2/3	[12]
HPV3	GP (Z) + NP (Z)	10 ⁶ 6	1	Rhesus	Zaire ^{***}	1/2	1/2	2/2	[12]
HPV3	GP (Z)	10 ⁶ 7	2	Rhesus	Zaire ^{***}	3/3	0/3	0/3	[12]
VLP	GP (Z) + NP (Z) + VP40 (Z)	250 µg	3	Cynomolgus	Zaire [*]	5/5	0/5	0/5	[13]

Abbreviations: Ad5-adenovirus serotype 5, E71D-substitution of aspartic for glutamic acid at position 71 of ZEBOV GP, GP-glycoprotein, GPΔ TM-recombinant GP lacking the transmembrane anchor region, IC-Ivory Coast ebolavirus, NP-nucleoprotein, NR-not reported, S-Sudan ebolavirus, VEEV-Venezuelan equine encephalitis virus, VLP-Virus-like particle, VP-virion protein, VSV-Vesicular stomatitis virus, Z-Zaire ebolavirus.

* Intramuscular.

** Aerosol.

*** Intraperitoneal.

Table 3

Approaches to postexposure filovirus vaccines in nonhuman primates (NHP)

System	Gene Product (Species or Strain)	Vaccine Dose	No. of Doses	Time post exposure	NHP Species	Challenge Species or Strain	Survivors/Total	Viremic/Total	Illness/Total	Ref
VSV	GP (MARV-Musoke)	10 ⁶ 7	1	20-30 min	Rhesus	MARV-Musoke	5/5	0/5	0/5	[64]
VSV	GP (MARV-Musoke)	10 ⁶ 7	1	1 day	Rhesus	MARV-Musoke	5/6	1/6	4/6	[67]
VSV	GP (MARV-Musoke)	10 ⁶ 7	1	2 days	Rhesus	MARV-Musoke	2/6	5/6	6/6	[67]
VSV	GP (Z)	10 ⁶ 7	1	20-30 min	Rhesus	ZEBOV	4/8	8/8	8/8	[65]
VSV	GP (S)	10 ⁶ 7	1	20-30 min	Rhesus	SEBOV	4/4	2/4	4/4	[66]

Abbreviations: GP-glycoprotein, S-Sudan ebolavirus, VSV-Vesicular stomatitis virus, Z-Zaire ebolavirus