Recombinant Vesicular Stomatitis Virus–Based Vaccines Against Ebola and Marburg Virus Infections

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The filoviruses, Marburg virus and Ebola virus, cause severe hemorrhagic fever with a high mortality rate in humans and nonhuman primates. Among the most-promising filovirus vaccines under development is a system based on recombinant vesicular stomatitis virus (rVSV) that expresses a single filovirus glycoprotein (GP) in place of the VSV glycoprotein (G). Importantly, a single injection of blended rVSV-based filovirus vaccines was shown to completely protect nonhuman primates against Marburg virus and 3 different species of Ebola virus. These rVSV-based vaccines have also shown utility when administered as a postexposure treatment against filovirus infections, and a rVSV-based Ebola virus vaccine was recently used to treat a potential laboratory exposure. Here, we review the history of rVSV-based vaccines and pivotal animal studies showing their utility in combating Ebola and Marburg virus infections.

Marburg virus (MARV) and Ebola virus (EBOV) are significant human pathogens that present a public health concern as emerging or reemerging viruses and as potential biological weapons. No vaccine or antiviral drug for MARV or EBOV is currently licensed and available for human use. Although Marburg and Ebola hemorrhagic fevers (HF) are rare diseases, a preventive vaccine could be important for several groups, including risk groups during filovirus outbreaks in endemic areas in sub-Saharan Africa (medical personnel, patient care personnel, family members); national and international healthcare workers and outbreak response personnel; laboratory workers conducting research on filoviruses; and military and other service personnel susceptible to filoviruses used as bioweapons. The properties of the filovirus vaccines required by these diverse groups may vary. For example, although laboratory and healthcare workers and some military personnel in stable settings with defined risk may be candidates for a multidose vaccine, outbreak settings require protection that is rapidly conferred with a single administration. The ideal vaccine that meets all needs would, with a single administration, rapidly confer long-term protection with little or no filovirus viremia against all species of EBOV that are pathogenic in humans as well as the diverse strains of MARV.

Remarkable progress has been made over the preceding decade in developing candidate preventive vaccines against filoviruses in nonhuman primate (NHP) models. There are at least 6 different vaccine systems that have shown promise in completely protecting NHPs against EBOV or MARV infection [1–16] (Table 1). Among these prospective vaccines that have shown efficacy in NHP models of filoviral HF, 2 candidates, 1 based on a replication-defective adenovirus serotype 5 and the other on recombinant vesicular stomatitis virus (rVSV), have provided complete protection to NHPs when administered as a single injection. This review focuses on rVSV-based filovirus vaccines.

Potential conflicts of interest: T. W. G. and H. F. claim intellectual property regarding vesicular stomatitis virus–based vaccines for Ebola and Marburg infections.

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Table 1. Most-Promising Vaccine Platforms With Efficacy in Nonhuman Primates

Platform	Immunogen	Characteristic	Preexposure (Efficacy)	Postexposure (Efficacy)	Concerns
Adenovirus, type 5 (Ad5)	GP; NP; GP/NP	Replication-deficient	Single vaccination (100%)	Not reported	Preexisting immunity; high dose
Virus-like particles (VLPs)	VP40/NP/GP	Replication-deficient	Multiple vaccinations (100%)	Not reported	Production; boost immunization
Venezuelan Equine Encephalitis virus (VEEV) replicon	GP; NP; GP/NP	Single round replication	Multiple vaccinations (0%–100%)	Not reported	Boost immunization
DNA	GP; NP	Replication-deficient	Multiple vaccinations (0%–100%)	Not reported	Boost immunization; efficacy questionable
Human parainfluenza virus, type 3 (HPIV3)	GP; GP/NP	Replication-competent (live-attenuated)	Single vaccination (50%100%)	Not reported	Preexisting immunity; safety
Vesicular stomatitis virus (VSV), Indiana serotype	GP	Replication-competent (live-attenuated)	Single vaccination (100%)	Single treatment (50%–100%)	Safety

NOTE. GP, glycoprotein; NP, nucleoprotein; VP40, virion protein 40 kDa.

EBOLA AND MARBURG VIRUSES

Marburg virus (MARV) and Ebola virus (EBOV), the causative agents of Marburg and Ebola HF, represent the 2 genera that comprise the family *Filoviridae* [17, 18]. The *Marburgvirus* genus contains a single species, *Lake Victoria marburgvirus* (MARV), whereas the *Ebolavirus* genus is comprised of 4 recognized species: (1) *Sudan ebolavirus* (SEBOV), (2) *Zaire ebolavirus* (ZEBOV), (3) *Côte d'Ivoire ebolavirus* (also known and here referred to as *Ivory Coast ebolavirus* (ICEBOV)), and (4) *Reston ebolavirus* (REBOV). A putative fifth species, *Bundigbugyo ebolavirus* (BEBOV), was associated with an outbreak in Uganda in 2007 [19]. 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 [18]). Based on a single outbreak, the newly discovered BEBOV appears to be less pathogenic, with a case-fatality rate of about 25% [19]. ICEBOV caused deaths in chimpanzees and a severe nonlethal human infection in a single case in the Republic of Côte d'Ivoire in 1994 [20]. REBOV is lethal for macaques but has not yet been reported to cause disease in humans [18].

EBOV and MARV are filamentous, enveloped, nonsegmented, negative-sense RNA viruses with genomes of approximately 19 kb. Each virus encodes 7 structural gene products in the following order: nucleoprotein (NP), virion protein (VP) 35, VP40, glycoprotein (GP), VP30, VP24, and the polymerase (L). In addition, EBOV expresses at least 1 nonstructural soluble GP (sGP) encoded by the GP gene [17, 18]. The GP, with variable contributions from other viral proteins such as NP, appears to be the key immunogenic protein in vaccine protection.

RECOMBINANT VSV AS A VACCINE VECTOR FOR FILOVIRUSES

VSV is the prototypic member of the family *Rhabdoviridae*, a family of negative-stranded RNA viruses with a simple genome organization encoding 5 structural proteins in the order nucleocapsid (N), phosphoprotein (P), matrix (M), glycoprotein (G), and an RNA-dependent RNA polymerase (L) [21]. VSV causes a disease in cattle, horses, deer, and pigs that is characterized by vesiculation and ulceration of the tongue, oral tissues, feet, and teats [21]. Infected animals typically recover within 2 weeks. Whereas naturally occurring VSV infection of humans is rare, infections have been reported in persons who were directly exposed to infected livestock, who were living within endemic regions, or who were accidentally exposed in laboratories [22–24]. VSV infection of humans usually either is asymptomatic or causes a mild influenza-like illness [22-24]. Among small animals, mice have shown utility as a model for evaluating VSV pathogenesis. For example, intranasal infection of mice with VSV results in significant weight loss (up to 20% of preinfection body weight) 2–5 days after infection [25, 26]. This weight loss is a convenient measure of VSV pathogenesis. Also, intracerebral inoculation of mice with VSV produces significant neuropathology that has shown utility in neurovirulence assays [27].

Previous studies demonstrated that rhabdoviruses have utility as expression vectors with potential use as viral vaccine vectors [25–32]. Live viral vaccines have traditionally offered the highest level of protection against viral infections. Such vaccines induce strong cellular and humoral host immune responses as a result of the intracellular synthesis of specific antigens at high levels over a prolonged period. In the last few years, Rose and colleagues have pioneered the use of rVSV, the prototypic member of the *Rhabdoviridae* family, as an expression and vaccine vector [25, 26, 28, 33–35]. Certain characteristics of VSV suggest that rVSVs expressing foreign viral genes would be good vaccine candidates. VSV grows to very high titers in many cell lines in vitro ($>10^9$ plaque-forming units [PFU]/mL) and can be propagated in almost all mammalian cells. VSV elicits strong humoral and cellular responses in vivo, and is able to elicit both mucosal and systemic immunity. Additionally, the extremely

low percentage of VSV seropositivity in the general population [22–24] and the lack of serious pathogenicity in humans are some of the possible advantages of using rVSV vaccines in humans. Importantly, the single-stranded RNA genome of VSV does not undergo reassortment and therefore lacks the potential to undergo genetic shifts in vivo. Furthermore, VSV replicates within the cytoplasm of infected cells and does not undergo genetic recombination.

More than a decade ago, a procedure for generating replication-competent, negative-stranded rVSV entirely from complementary DNA was established [34]. The genetic flexibility of VSV has allowed the development of rVSVs that express foreign viral proteins to high levels [33, 36]. Several different strategies have been employed in developing candidate replication-competent rVSV-based vaccines, all of which have adopted measures to enhance safety. For example, previous studies have shown that the pathogenesis and neurovirulence of VSV in mice is directly associated with the VSV G [26, 37, 38]. Studies have also shown that the VSV G is the determinant of pathogenesis in swine [39]. To attenuate rVSV vectors, some groups have made mutations truncating the VSV G cytoplasmic domain from 29 to 9 or 1 amino acid. This action resulted in abrogating pathogenesis in mice [26]. Another approach has been to develop GP exchange vectors where the VSV G is completely deleted and replaced with a foreign GP [36].

The generation of rVSVs and their utility in preventing virus infections was shown in several studies. Rose and colleagues demonstrated that live attenuated rVSV expressing the human immunodeficiency virus (HIV) envelope (env) and core (Gag) proteins protected rhesus monkeys from AIDS following challenge with a pathogenic AIDS virus [28]. Similarly, Roberts and coworkers developed rVSV vectors expressing influenza hemagglutinin (HA) protein, which are completely attenuated for pathogenesis in the mouse model [25]. This nonpathogenic vaccine also provided complete protection from lethal influenza virus challenge. Moreover, another vaccine with the VSV G deleted and expressing HA (rVSV△G-HA) was also protective and nonpathogenic, and had the additional advantage of inducing no neutralizing antibody to the vector itself [25]. Importantly, mice immunized with the rVSV△G-HA and subsequently challenged with wild-type VSV develop neutralizing antibody titers to VSV; these antibodies are directed against VSV G. It was subsequently shown that these GP exchange vectors allowed effective boosting and generation of neutralizing antibodies to a primary isolate of HIV type 1 [28]. Together, these studies indicate that rVSV△G is a reusable vector, which is a particularly important advantage in any vaccine platform.

Using the strategy shown for developing nonpathogenic rVSV△G vectors expressing influenza genes, rVSV△G vaccines were developed for EBOV and MARV [4, 12, 40]. The rVSV△G vectors were modified to carry the GP gene from ZEBOV, SEBOV, or the Musoke strain of MARV in place of the VSV G protein. All rVSV△G viruses expressing filovirus GPs exhibited rhabdovirus morphology. Unlike the rVSVs with an additional transcription unit expressing the soluble GPs, the viruses carrying the foreign transmembrane filovirus GPs in replacement of the VSV G were slightly attenuated in growth [40].

RECOMBINANT VSV VECTORS AS PREVENTIVE FILOVIRUS VACCINES

Initial studies using rVSV△G-based filovirus vaccines focused on the ability of these vaccines to protect animals against homologous filovirus challenges. Vaccination of BALB/c mice with a single intraperitoneal injection of as few as 2 PFU of a rVSV△G vector expressing the ZEBOV GP completely protected the animals against a lethal mouse-adapted ZEBOV challenge 28 days after the immunization [41]. Likewise, a single intramuscular vaccination of cynomolgus monkeys with a rVSV△G vector expressing the ZEBOV GP induced strong humoral and cellular immune responses in vaccinated monkeys and elicited complete protection against a high-dose (1000 PFU) intramuscular challenge of homologous ZEBOV given 28 days later [4] (Table 2). However, there was no cross-protection, as subsequent back-challenge of the ZEBOV-surviving macaques with SEBOV resulted in fatal disease [4]. This rVSV△G ZEBOV vaccine was subsequently tested against a high-dose (1000 PFU) aerosol challenge of homologous ZEBOV. A single intramuscular vaccination of cynomolgus monkeys with rVSV△G ZEBOV GP completely protected animals against a homologous aerosol challenge of ZEBOV given 28 days later [9] (Table 2). Furthermore, protection can be conferred by these rVSV△G ZEBOV GP vaccines via various delivery routes; immunization of either BALB/c mice or cynomolgus monkeys with the rVSV△G ZEBOV GP vaccine by either the intranasal or oral route resulted in complete protection of all animals against a high-dose (1000 PFU) intramuscular homologous ZEBOV challenge [13, 41] (Table 2). Robust ZEBOV GP-specific humoral responses and T-cell responses were induced after vaccination and 6 months after ZEBOV challenge in macaques vaccinated by either the intranasal or oral route [13].

For MARV, a single intramuscular vaccination of cynomolgus monkeys with a rVSV△G MARV-Musoke GP vaccine elicited complete protection against a high-dose (1000 PFU) intramuscular challenge of homologous MARV given 28 days later [4] (Table 2). The animals were also protected on rechallenge with a 1967 MARV strain 113 days later [4]. Furthermore, the same vaccine proved protective against the genetically most-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 [5] (Table 2). As with ZEBOV, recent studies showed that a single vaccination of cynomolgus monkeys with rVSV△G MARV-Musoke GP

Table 2. Preventive Recombinant Vesicular Stomatitis Virus–Based Ebola and Marburg Virus Vaccines in Nonhuman Primates

Gene product (species or strain)	Route of vaccine	Vaccine dose, PFU	No. of doses	NHP species	Challenge species	Survivors, n/Total, n	Viremic, n/Total, n	Illness, n/Total, n	Reference
GP (Z)	IM	10^7	1	Cynomolgus	EBOV-Zaire[a]	4/4	0/4	0/4	[4]
GP (Z)	IM	10^7	1	Cynomolgus	EBOV-Zaire[a]	2/2	0/2	0/2	[13]
GP (Z)	IM	10^7	1	Cynomolgus	EBOV-Zaire[b]	3/3	0/3	0/3	[9]
GP (Z) + GP (S) + GP (M-Musoke)	IM	10^7	1	Cynomolgus	EBOV-Zaire[a]	3/3	0/3	0/3	[12]
GP (Z) + GP (S) + GP (M-Musoke)	IM	10^7	1	Cynomolgus	EBOV-Sudan[a]	2/2	0/2	0/2	[12]
GP (Z) + GP (S) + GP (M-Musoke)	IM	10^7	1	Cynomolgus	EBOV-Ivory Coast[a]	3/3	0/3	0/3	[12]
GP (Z)	IM	10^7	1	Cynomolgus	EBOV-Sudan[a]	0/1	1/1	1/1	[12]
GP (Z) + GP (S) + GP (M-Musoke)	IM	10^7	2	Rhesus	EBOV-Sudan[a]	3/3	0/3	0/3	[12]
GP (Z)	oral	10^7	1	Cynomolgus	EBOV-Zaire[a]	4/4	NR	0/4	[13]
GP (Z)	IN.	10^7	1	Cynomolgus	EBOV-Zaire[a]	4/4	NR	0/4	[13]
GP (M-Musoke)	IM	10^7	1	Cynomolgus	MARV-Musoke[a]	4/4	0/4	0/4	[4]
GP (M-Musoke)	IM	10^7	1	Cynomolgus	MARV-Musoke[a]	1/1	0/1	0/1	[5]
GP (M-Musoke)	IM	10^7	1	Cynomolgus	MARV-Ravn[a]	3/3	0/3	0/3	[5]
GP (M-Musoke)	IM	10^7	1	Cynomolgus	MARV-Angola[a]	3/3	0/3	0/3	[5]
GP (M-Musoke)	IM	10^7	1	Cynomolgus	MARV-Musoke[b]	4/4	0/4	0/4	[9]
GP (Z) + GP (S) + GP (M-Musoke)	IM	10^7	1	Cynomolgus	MARV-Musoke[a]	3/3	0/3	0/3	[12]

NOTE. PFU, plaque-forming units; NHP, nonhuman primate; GP, glycoprotein; IM, intramuscular; IN, intranasal; M, *Lake Victoria marburgvirus* (MARV); NP, nucleoprotein; NR, not reported, S, *Sudan ebolavirus*; VP, virion protein; Z, *Zaire ebolavirus*.

[a] intramuscular.

[b] aerosol.

completely protected animals against a homologous aerosol challenge of MARV given 28 days later [9] (Table 2).

Because of 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 blended vaccine consisting of equal parts of the rVSV△G GP vaccines for MARV, EBOV, and SEBOV [12]. At 4 weeks post-vaccination, groups of the animals were challenged with MARV, ZEBOV, SEBOV, or ICEBOV (Table 2). None of the vaccinated macaques succumbed to a filovirus challenge, showing great promise with this single-injection, multivalent, blended platform.

RECOMBINANT VSV–BASED FILOVIRUS VACCINES AS TREATMENTS

In addition to its utility as a preventive vaccine, the rVSV△G vaccine platform has also been used as a postexposure treatment for filovirus infections [Table 3]. Treatment of rhesus monkeys with rVSV△G MARV-Musoke GP shortly after a homologous high-dose MARV challenge resulted in complete protection of all animals from clinical illness and death [42]. Subsequent studies demonstrated that the rVSV△G GP vaccines for ZEBOV and SEBOV protected 50% and 100%, respectively, of rhesus macaques when administered as postexposure prophylaxis after high-dose homologous virus challenge [43, 44]. The vaccine was administered in these studies 20–30 minutes after filovirus challenge. A major question is how long after virus exposure can the rVSV△G vaccine be effective? In a recent study, treatment of rhesus monkeys with rVSV△G MARV-Musoke GP 24 hours after homologous MARV challenge resulted in protection of 5 of 6 monkeys, whereas, remarkably, 2 of 6 animals were protected when the vaccine was administered 48 hours after infection [45].

SAFETY OF RECOMBINANT VSV–BASED FILOVIRUS VACCINES

The main concern with all replication-competent vaccines, including the rVSV△G platform, is their safety, especially in persons with compromised immune systems. However, initial results of various rVSV△G and rVSV vectors in NHPs are promising. No toxicity was seen in rhesus macaques following intranasal inoculation with wild-type VSV, rVSV, and 2 rVSV-HIV vaccines, although significant neurovirulence was noted in 1 of 4 animals after direct intrathalamic inoculation with rVSV [46]. To date, no toxicity has been seen in >80 NHPs given rVSV△G MARV or EBOV vaccines [4, 5, 9, 12, 13, 47].

Table 3. Postexposure Recombinant Vesicular Stomatitis Virus–Based Ebola and Marburg Virus Vaccines in Nonhuman Primates

Gene product (species or strain)	Vaccine dose, PFU	No. of doses	Time postexposure	NHP species	Challenge species or strain	Survivors, n/Total, n	Viremic, n/Total, n	Illness, n/Total, n	Reference
GP (M-Musoke)	10^7	1	20–30 min	Rhesus	MARV-Musoke	5/5	0/5	0/5	[42]
GP (M-Musoke)	10^7	1	1 d	Rhesus	MARV-Musoke	5/6	1/6	4/6	[45]
GP (M-Musoke)	10^7	1	2 d	Rhesus	MARV-Musoke	2/6	5/6	6/6	[45]
GP (Z)	10^7	1	20–30 min	Rhesus	EBOV-Zaire	4/8	8/8	8/8	[43]
GP (S)	10^7	1	20–30 min	Rhesus	EBOV-Sudan	4/4	2/4	4/4	[44]

NOTE. PFU, plaque-forming units; NHP, nonhuman primate; GP, glycoprotein; M, *Lake Victoria marburgvirus* (MARV); S, *Sudan ebolavirus*; Z, *Zaire ebolavirus*.

Furthermore, no significant vaccine shedding has been seen in these experiments despite challenge doses of up to 10^7 PFU [4, 5, 9, 12, 13, 47] which suggests, along with the natural low transmissibility of VSV [48], that spread to persons outside the vaccine target population is unlikely.

To specifically address safety of the rVSV△G-based filovirus vaccines, the rVSV△G ZEBOV GP vaccine was evaluated in 2 animal models with defective immune systems, nonobese diabetic–severe combined immunodeficient (NOD-SCID) mice [41] and simian/human immunodeficiency (SHIV) –infected rhesus monkeys [47]. On immunization, no evidence of overt illness was noted in any of the animals. In addition, the rVSV△G ZEBOV GP vaccine was recently used to treat a laboratory worker after a recent laboratory accident [49]. The vaccine was administered <48 hours after potential ZEBOV exposure. The patient developed fever, headache, and myalgia 12 hours after injection. 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. To further ensure safety, the VSV G, to which the pathogenicity of wild-type VSV may be attributable [37–39] has been deleted in the rVSV△G filovirus vaccines. Current studies are being performed to evaluate the neurovirulence of the rVSV△G ZEBOV GP and rVSV△G MARV-Musoke vaccines in a conventional intrathalamic neurovirulence assay in cynomolgus monkeys. Regarding possible vaccine virus mutation to more-virulent variants, some comfort can be taken from noting the case of the live measles vaccine that has been in use in the United States and other countries since the 1960s (reviewed in [50]) with no evidence of acquiring a higher pathogenic potential.

SUMMARY

Vaccine development for EBOV and MARV has been successful over the preceding decade and has generated several promising experimental approaches (Table 1). The rVSV platform has shown complete efficacy as a preventive single-shot vaccine in 3 relevant animal models, including the ''gold standard'' nonhuman primate models. A blended cocktail has shown complete efficacy as a preventive vaccine against all public health relevant filovirus species with partial overlapping endemicity zones in central Africa. Finally, the platform has shown partial to complete efficacy in postexposure treatment against homologous filovirus challenge. Given the efficacy profile in preventive and treatment approaches and the safety record in several immune-competent and immune-compromised animal species, this vaccine platform is ready to be considered for investigational drug licensure. We further propose to consider the rVSV platform for preinvestigational drug use in cases of laboratory exposures with EBOV and MARV. In addition to EBOV and MARV, rVSV-based vaccines are currently being evaluated for a number of other human pathogens, including avian influenza, hepatitis B, HIV, Lassa fever, severe acute respiratory syndrome (SARS), West Nile virus, and *Yersinia pestis*. Knowledge gained from these studies should advance the development of rVSV-based vaccines for human use.

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