

Chapter 2

Viral-Vectored Vaccines to Control Pathogenic Filoviruses

Chad E. Mire and Thomas W. Geisbert

Abstract For more than 35 years the filoviruses, Marburg virus and Ebola virus, have caused sporadic outbreaks of hemorrhagic fever that result in severe and often fatal disease in humans and nonhuman primates. Pathogenic Marburg and Ebola viruses are endemic in resource-poor regions in Central Africa and are also of concern as they have the potential for deliberate misuse. Although no vaccines or antiviral drugs for filoviruses are currently available for human use, remarkable progress has been made in developing candidate preventive vaccines against Marburg and Ebola viruses in nonhuman primate models. Most of these vaccines are based on viral vectors including recombinant adenoviruses, alphaviruses, paramyxoviruses, and rhabdoviruses. Because of the remote geographic locations of most filovirus outbreaks, a single-injection vaccine is an important goal in vaccine development. Among the prospective viral-vectored vaccines that have demonstrated 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 vesicular stomatitis virus (rVSV), were shown to confer complete protection to nonhuman primates when administered as a single injection. Notably, the rVSV-based vaccines have also shown utility when used as postexposure treatments for filovirus infections.

2.1 Introduction

Marburg virus (MARV) and Ebola virus (EBOV), the causative agents of Marburg and Ebola hemorrhagic fever (HF), comprise the family *Filoviridae* (Feldmann et al. 2013). The taxonomy of filoviruses has become complicated and controversial. For the purposes of this chapter, the most commonly used terms in the

C.E. Mire • T.W. Geisbert (✉)

Galveston National Laboratory, University of Texas Medical Branch, 301 University Blvd.,
Galveston, TX 77550-0610, USA

Department of Microbiology and Immunology, University of Texas Medical Branch,
Galveston, TX, USA

e-mail: twgeisbe@utmb.edu

published literature will be used to best ensure clarity. The MARV genus contains two lineages: one represented by a number of strains including Angola, Ci67, and Popp and a second lineage represented by the Ravn strain. The EBOV genus is comprised of five distinct species: (1) *Sudan ebolavirus* (SEBOV), (2) *Zaire ebolavirus* (ZEBOV), (3) *Ivory Coast ebolavirus* (ICEBOV) (also referred to as *Cote d'Ivoire ebolavirus* or *Tai Forest ebolavirus*), (4) *Bundibugyo ebolavirus* (BEBOV), and (5) *Reston ebolavirus* (REBOV) (Feldmann et al. 2013). MARV, ZEBOV, SEBOV, and BEBOV are important human pathogens with case fatality rates frequently ranging up to 90 % for MARV and ZEBOV, around 50–55 % for SEBOV, and 40–48 % for BEBOV [reviewed in Feldmann et al. (2013)]. ICEBOV caused mortality in chimpanzees and a severe nonlethal human infection in a single case in the Republic of Cote d'Ivoire in 1994 (Le Guenno et al. 1995). REBOV is highly lethal for cynomolgus macaques but has not been associated with disease in humans (Feldmann et al. 2013). An outbreak of REBOV was reported in 2008 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 coinfecting the animals, especially porcine reproductive and respiratory syndrome virus (Barrette et al. 2009).

Filoviruses are filamentous enveloped non-segmented negative-sense RNA viruses with genomes approximately 19 kb in length. These viruses encode seven gene products: the nucleoprotein (NP), virion protein (VP)35, VP40, glycoprotein (GP), VP30, VP24, and polymerase (L). In addition, the EBOV species express two additional nonstructural proteins from the GP gene referred to as soluble (s)GP and small soluble (ss)GP [reviewed in Feldmann et al. (2013)].

Currently, there are no FDA-approved vaccines or postexposure treatments available for preventing or managing EBOV or MARV infections; however, there are at least seven 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 (Hevey et al. 1998; Sullivan et al. 2000, 2003, 2006, 2011; Jones et al. 2005; Daddario-DiCaprio et al. 2006a; Bukreyev et al. 2007; Warfield et al. 2007; Geisbert et al. 2008a, 2009, 2010a, 2011; Swenson et al. 2008a, b; Qiu et al. 2009; Pratt et al. 2010; Hensley et al. 2010, 2013; Falzarano et al. 2011; Blaney et al. 2013; Marzi et al. 2013; Richardson et al. 2013; Mire et al. 2013). A preventive vaccine would be 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 endemic regions, (3) personnel involved in outbreak response missions, (4) laboratory workers conducting research on filoviruses, and (5) military and other service personnel susceptible to the use of filoviruses as biological weapons.

The requirements for a filovirus vaccine may vary based on the diversity of the affected populations. While multidose vaccine regimens would be feasible for laboratory and healthcare workers and some military personnel in stable settings with defined risk, an outbreak setting or a case of deliberate release would require

rapidly conferred protection with a single administration. The durability of protection required by each group may also vary. Laboratory or healthcare workers and military personnel rotating through high-risk situations for fixed periods may not require an extended 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 SEBOV, ZEBOV, BEBOV, and the diverse strains of MARV with a single inoculation.

2.2 Animal Models

Guinea pigs, mice, and hamsters have been used as animal models of filoviral HF (Bechtelsheimer et al. 1971; Zlotnik 1971; Ryabchikova et al. 1996; Bray et al. 1998, 2001; Connolly et al. 1999; Geisbert et al. 2002; Warfield et al. 2009; Ebihara et al. 2013). However, filovirus isolates derived from humans or NHPs do not typically produce severe disease in rodents upon initial exposure. Lethal infection requires serial adaptation with up eight or more passes in rodents in some cases. Guinea pigs and mice have served well as early screens for evaluating antiviral drugs and candidate vaccines, with genetically engineered mice providing a platform 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 (Bray et al. 2001; Geisbert et al. 2002). Some examples include the following: the coagulation disorders that are hallmark features of disease in filovirus-infected humans and NHPs are not present in filovirus-infected mice or guinea pigs (Bray et al. 2001; Geisbert et al. 2002), and while the bystander death of large numbers of uninfected lymphocytes due to apoptosis has been reported in filovirus-infected humans (Baize et al. 1999), NHPs (Geisbert et al. 2000), and mice (Bradfute et al. 2007), the morphology and process of lymphocyte apoptosis in primates and mice are not similar (Bradfute et al. 2007). A recently described hamster model of ZEBOV infection showed more similarity with primate disease than mice or guinea pigs (Ebihara et al. 2013), and further studies need to be conducted to fully assess the utility of this model. As data derived from studies using rodents may not correlate with human disease and as it is uncertain whether studies performed in rodents would be suitable for supporting applications for licensure of filovirus vaccines, this review focuses on vaccine studies performed in NHPs.

2.3 Viral-Vectored Vaccines

Recent efforts to develop vaccines for the filoviral HFs have focused on the use of various recombinant vectors expressing filovirus proteins to induce protective immunity (Tables 2.1, 2.2, 2.3, 2.4, 2.5 and 2.6). The delivery systems used for

Table 2.1 Preventive Marburg virus vaccines in nonhuman primates (NHP)

System	Gene product (strain)	Vaccine dose	No. of doses	NHP species	Challenge strain	Survivors/total	Viremic/Total	Illness/Total	References
VEEV replicon	GP (Musoke)	10^7	3	Cynomolgus	Musoke ^a	3/3	0/3	0/3	Hevey et al. (1998)
VEEV replicon	GP (Musoke) + NP (Musoke)	10^7	3	Cynomolgus	Musoke ^a	3/3	0/3	0/3	Hevey et al. (1998)
VEEV replicon	NP (Musoke)	10^7	3	Cynomolgus	Musoke ^a	2/3	3/3	3/3	Hevey et al. (1998)
VEEV replicon	GP (Musoke)	10^7	3	Cynomolgus	Ravn ^a	0/3	NR	3/3	Lundstrom (2003)
VEEV replicon	GP (Musoke) + NP (Musoke)	10^7	3	Cynomolgus	Ravn ^a	0/3	NR	3/3	Lundstrom (2003)
DNA prime	GP (Angola) for both	4 mg	3	Cynomolgus	Angola ^a	4/4	0/4	2/4	Geisbert et al. (2010a)
Ad5 boost		10^{11}	1						
Ad5	GP (Angola)	10^{11}	1	Cynomolgus	Angola ^a	4/4	0/4	0/4	Geisbert et al. (2010a)
Ad5	GP (Z) + NP (Z) + GP (S) + GP (Ci67) + GP (Ravn)	10^{10}	2	Cynomolgus	Musoke ^a	5/5	NR	1/5	Swenson et al. (2008a)
VSV	GP (Musoke)	10^7	1	Cynomolgus	Musoke ^a	4/4	0/4	0/4	Jones et al. (2005)
VSV	GP (Musoke)	10^7	1	Cynomolgus	Musoke ^a	1/1	0/1	0/1	Daddario-DiCaprio et al. (2006a)
VSV	GP (Musoke)	10^7	1	Cynomolgus	Ravn ^a	3/3	0/3	0/3	Daddario-DiCaprio et al. (2006a)
VSV	GP (Musoke)	10^7	1	Cynomolgus	Angola ^a	3/3	0/3	0/3	Daddario-DiCaprio et al. (2006a)

VSV	GP (Musoke)	10 ⁷	1	Cynomolgus	Musoke ^b	4/4	0/3	0/3	Geisbert et al. (2008a)
VSV	GP (Z) + GP (S) + GP (M-Musoke)	10 ⁷	1	Cynomolgus	Musoke ^a	3/3	0/3	0/3	Geisbert et al. (2009)

Ad5 adenovirus serotype 5, *GP* glycoprotein, *NP* nucleoprotein, *NR* not reported, *S Sudan Ebola virus*, *VEEV* Venezuelan equine encephalitis virus, *VSV* vesicular stomatitis virus, *Z Zaire Ebola virus*

^aIntramuscular

^bAerosol

Table 2.2 Preventive adenovirus-based 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	References
DNA prime	GP (Z) + GP (S) + GP (IC) + NP (Z)	4 mg	3	Cynomolgus	Zaire ^a	4/4	1/4	0/4	Sullivan et al. (2000)
Ad5 boost	GP(Z)	10 ¹⁰	1						
DNA prime	GP (Z) + GP (S)	4 mg	4	Cynomolgus	Bundibugyo ^a	4/4	1/4	1/4	Hensley et al. (2010)
Ad5 boost	GP (Z)	10 ¹¹	1						
Ad5	GP (Z) + NP (Z)	10 ¹²	1	Cynomolgus	Zaire ^a	4/4	0/4	0/4	Sullivan et al. (2003)
Ad5	GP (Z) + NP (Z)	10 ¹²	2	Cynomolgus	Zaire ^a	4/4	0/4	0/4	Sullivan et al. (2003)
Ad5	GP (Z) + NP (Z)	10 ¹²	1	Cynomolgus	Zaire ^a	4/4	0/4	0/4	Sullivan et al. (2006)
Ad5	GP (Z) + NP (Z)	10 ¹¹	1	Cynomolgus	Zaire ^a	3/3	0/3	0/3	Sullivan et al. (2006)
Ad5	GP (Z) + NP (Z)	10 ¹¹	2	Cynomolgus	Zaire ^a	3/3	0/3	0/3	Sullivan et al. (2011)
Ad5	GP (Z) + NP (Z)	10 ¹⁰	1	Cynomolgus	Zaire ^a	6/6	0/6	0/6	Sullivan et al. (2006)
Ad5	GP (Z) + NP (Z)	10 ⁹	1	Cynomolgus	Zaire ^a	0/3	3/3	3/3	Sullivan et al. (2006)
Ad5	GP (Z) + GP (S)	10 ¹⁰	2	Cynomolgus	Zaire ^a	1/1	0/1	0/1	Geisbert et al. (2011)
Ad5	GPΔTM (Z) + NP (Z)	10 ¹²	1	Cynomolgus	Zaire ^a	2/3	1/3	1/3	Sullivan et al. (2006)
Ad5	GPΔTM (Z) + NP (Z)	10 ¹¹	1	Cynomolgus	Zaire ^a	1/3	2/3	2/3	Sullivan et al. (2006)
Ad5	GP (Z) E71D + GP (S) E71D + NP (Z)	10 ¹⁰	1	Cynomolgus	Zaire ^a	1/3	2/3	2/3	Sullivan et al. (2006)
Ad5	GP (Z) E71D + GP (S) E71D	10 ¹⁰	1	Cynomolgus	Zaire ^a	3/3	0/3	0/3	Sullivan et al. (2006)
Ad5	GP (Z) E71D + NP (Z)	10 ¹⁰	1	Cynomolgus	Zaire ^a	2/3	1/3	1/3	Sullivan et al. (2006)
Ad5	GP (Z) + NP (Z) + GP (S) + GP (M-C167) + GP (M-Ravn)	10 ¹⁰	2	Cynomolgus	Zaire ^a	5/5	NR	0/5	Swenson et al. (2008a), Pratt et al. (2010)

Ad5	GP (Z) + NP (Z) + GP (S) + GP (M-C167) + GP (M-Ravn)	10 ¹⁰	2	Cynomolgus	Sudan ^a	5/5	NR	0/5	Pratt et al. (2010)
Ad5	GP (Z) + NP (Z) + GP (S)	10 ¹⁰	1	Cynomolgus	Zaire ^b	3/3	NR	0/3	Pratt et al. (2010)
Ad5	GP (Z) + NP (Z) + GP (S)	10 ¹⁰	1	Cynomolgus	Sudan ^b	2/3	NR	2/3	Pratt et al. (2010)
Ad5	GP (Z) + NP (Z) + GP (S)	10 ¹⁰	2	Cynomolgus	Sudan ^b	3/3	0/3	1/3	Pratt et al. (2010)
Ad5/ Ad- IFN α	GP (Z)	10 ¹⁰	1	Cynomolgus	Zaire ^a	3/3	NR	0/3	Richardson et al. (2013)
Ad5/ Ad- IFN α	GP (Z) (i.n. + i.t.)	10 ¹⁰	1	Cynomolgus	Zaire ^a	2/3	NR	3/3	Richardson et al. (2013)
Ad26	GP (Z) + GP (S)	10 ¹²	1	Cynomolgus	Zaire ^a	3/4	1/4	1/4	Geisbert et al. (2011)
Ad35	GP (Z)	10 ¹⁰	1	Cynomolgus	Zaire ^a	1/6	5/6	6/6	Geisbert et al. (2011)
Ad35	GP (Z)	10 ¹¹	1	Cynomolgus	Zaire ^a	0/3	3/3	3/3	Geisbert et al. (2011)
Ad26/ Ad35	GP (Z) + GP (S)	10 ¹¹	2	Cynomolgus	Zaire ^a	4/4	0/4	0/4	Geisbert et al. (2011)

Ad5 adenovirus serotype 5, E71D substitution of aspartic for glutamic acid at position 71 of ZEBOV GP, GP glycoprotein, GP Δ TM recombinant GP lacking the transmembrane anchor region, IC Ivory Coast Ebola virus, NP nucleoprotein, NR not reported, S Sudan Ebola virus, Z Zaire Ebola virus

^aIntramuscular

^bAerosol

Table 2.3 Preventive adenovirus-based Ebola virus vaccines in adenovirus-immune nonhuman primates (NHP)

System	Gene product (species)	Vaccine dose	Vaccine route	NHP species	Challenge species	Survivors/ total	Viremic/ total	Illness/ total	References
Ad5	GP (Z)	10 ¹⁰	i.m.	Cynomolgus	Zaire ^a	0/3	3/3	3/3	Geisbert et al. (2011)
Ad26	GP (Z) + GP (S)	10 ¹⁰	i.m.	Cynomolgus	Zaire ^a	0/4	4/4	4/4	Geisbert et al. (2011)
Ad26	GP (Z) + GP (S)	10 ¹¹	i.m.	Cynomolgus	Zaire ^a	2/4	2/4	2/4	Geisbert et al. (2011)
Ad35	GP (Z)	10 ¹⁰	i.m.	Cynomolgus	Zaire ^a	1/3	2/3	2/3	Geisbert et al. (2011)
Ad5/Ad-IFN α	GP (Z)	10 ¹⁰	i.m.	Cynomolgus	Zaire ^a	0/3	NR	3/3	Richardson et al. (2013)
Ad5/Ad-IFN α	GP (Z)	10 ¹⁰	i.n./i.t.	Cynomolgus	Zaire ^a	3/4	NR	4/4	Richardson et al. (2013)

Ad5 adenovirus serotype 5, Ad26 adenovirus serotype 26, Ad35 adenovirus serotype 35, NR not reported, S Sudan Ebola virus, Z Zaire Ebola virus
^aIntramuscular

Table 2.4 Preventive vesicular stomatitis virus-based 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	References
VSV	GP (Z)	10^7	1	Cynomolgus	Zaire ^a	4/4	0/4	0/4	Jones et al. (2005)
VSV	GP (Z)	10^7	1	Cynomolgus	Zaire ^a	4/4	0/4	0/4	Marzi et al. (2013)
VSV	GP (Z)	10^7	1	Cynomolgus	Zaire ^b	3/3	0/3	0/3	Geisbert et al. (2008a)
VSV	GP (Z) + GP (S) + GP (M-Musoke)	10^7	1	Cynomolgus	Zaire ^a	3/3	0/3	0/3	Geisbert et al. (2009)
VSV	GP (Z) + GP (S) + GP (M-Musoke)	10^7	1	Cynomolgus	Sudan ^a	2/2	0/2	0/2	Geisbert et al. (2009)
VSV	GP (Z) + GP (S) + GP (M-Musoke)	10^7	1	Cynomolgus	Ivory Coast ^a	3/3	0/3	0/3	Geisbert et al. (2009)
VSV	GP (Z)	10^7	1	Cynomolgus	Sudan ^a	0/1	1/1	1/1	Geisbert et al. (2009)
VSV	GP (Z) + GP (S) + GP (M-Musoke)	10^7	2	Rhesus	Sudan ^a	3/3	0/3	0/3	Geisbert et al. (2009)
VSV	GP (Z) – oral	10^7	1	Cynomolgus	Zaire ^a	4/4	NR	0/4	Qiu et al. (2009)
VSV	GP (Z) – IN	10^7	1	Cynomolgus	Zaire ^a	4/4	NR	0/4	Qiu et al. (2009)
VSV	GP (IC)	10^7	1	Cynomolgus	Bundibugyo ^a	1/3	3/3	3/3	Falzarano et al. (2011)
VSV	GP (Z)	10^7	1	Cynomolgus	Bundibugyo ^a	3/4	4/4	4/4	Falzarano et al. (2011)
VSV	GP (B)	10^7	1	Cynomolgus	Bundibugyo ^a	3/3	0/3	0/3	Mire et al. (2013)

(continued)

Table 2.4 (continued)

System	Gene product (species)	Vaccine dose	No. of doses	NHP species	Challenge species	Survivors/total	Viremic/total	Illness/total	References
VSV	GP (Z) + GP (S)	10 ⁷	1	Cynomolgus	Bundibugyo ^a	1/3	3/3	3/3	Mire et al. (2013)
VSV	GP (Z) + GP (S)	10 ⁷	2	Cynomolgus	Bundibugyo ^a	3/3	0/3	0/3	Mire et al. (2013)

GP glycoprotein, B Bundibugyo Ebola virus, IC Ivory Coast Ebola virus, NR not reported, S Sudan Ebola virus, VSV vesicular stomatitis virus, Z Zaire Ebola virus

^aIntramuscular

^bAerosol

Table 2.5 Other 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	References
Vaccinia	GP (Z)	10^7	3	Cynomolgus	Zaire ^a	0/3	3/3	3/3	Geisbert et al. (2002)
VEEV replicon	GP (Z)	10^7	3	Cynomolgus	Zaire ^a	0/3	3/3	3/3	Geisbert et al. (2002)
VEEV replicon	NP (Z)	10^7	3	Cynomolgus	Zaire ^a	0/3	3/3	3/3	Geisbert et al. (2002)
VEEV replicon	GP (Z) + NP (Z)	10^7	3	Cynomolgus	Zaire ^a	0/3	3/3	3/3	Geisbert et al. (2002)
VEEV replicon	GP (S)	10^{10}	1	Cynomolgus	Sudan ^a	6/6	0/6	6/6	Herbert et al. (2013)
VEEV replicon	GP (S) + GP (Z)	10^{10}	1	Cynomolgus	Sudan ^a	3/3	0/3	0/3	Herbert et al. (2013)
VEEV replicon	GP (S) + GP (Z)	10^{10}	1	Cynomolgus	Zaire ^a	3/3	0/3	0/3	Herbert et al. (2013)
VEEV replicon	GP (S)	10^{10}	1	Cynomolgus	Sudan ^b	0/3	2/3	3/3	Herbert et al. (2013)
VEEV replicon	GP (S)	10^{10}	2	Cynomolgus	Sudan ^b	3/3	1/3	2/3	Herbert et al. (2013)
HPIV3	GP (Z)	10^6	1	Rhesus	Zaire ^c	4/4	0/4	1/4	Bukreyev et al. (2007)
HPIV3	GP (Z)	10^7	1	Rhesus	Zaire ^c	2/3	2/3	2/3	Bukreyev et al. (2007)
HPIV3	GP (Z) + NP (Z)	10^6	1	Rhesus	Zaire ^c	1/2	1/2	2/2	Bukreyev et al. (2007)
HPIV3	GP (Z)	10^7	2	Rhesus	Zaire ^c	3/3	0/3	0/3	Bukreyev et al. (2007)

(continued)

Table 2.5 (continued)

System	Gene product (species)	Vaccine dose	No. of doses	NHP species	Challenge species	Survivors/ total	Viremic/ total	Illness/ total	References
RABV-RC	GP (Z)	10 ⁷	1	Rhesus	Zaire ^a	4/4	1/4	4/4	Blaney et al. (2013)
RABV-RD	GP (Z)	10 ⁷	1	Rhesus	Zaire ^a	2/4	3/4	4/4	Blaney et al. (2013)

GP glycoprotein, NP nucleoprotein, S Sudan Ebola virus, RABV-RC replication-competent rabies virus, RABV-RD replication-defective rabies virus, VEEV Venezuelan equine encephalitis virus, Z Zaire Ebola virus

^aIntramuscular

^bAerosol

^cIntraperitoneal

Table 2.6 Postexposure filovirus vaccines in nonhuman primates (NHP)

System	Gene product (species or strain)	Vaccine dose	No. of doses	Time postexposure	NHP species	Challenge species or strain	Survivors/ total	Viremic/ total	Illness/ total	References
VSV	GP (MARV-Musoke)	10 ⁷	1	20–30 min	Rhesus	MARV-Musoke	5/5	0/5	0/5	Daddario-DiCaprio et al. (2006b)
VSV	GP (MARV-Musoke)	10 ⁷	1	1 day	Rhesus	MARV-Musoke	5/6	1/6	4/6	Geisbert et al. (2010b)
VSV	GP (MARV-Musoke)	10 ⁷	1	2 days	Rhesus	MARV-Musoke	2/6	5/6	6/6	Geisbert et al. (2010b)
VSV	GP (Z)	10 ⁷	1	20–30 min	Rhesus	ZEBOV	4/8	8/8	8/8	Feldmann et al. (2007)
VSV	GP (S)	10 ⁷	1	20–30 min	Rhesus	SEBOV	4/4	2/4	4/4	Geisbert et al. (2008b)

GP glycoprotein, S Sudan Ebola virus, VSV vesicular stomatitis virus, Z Zaire Ebola virus

these purposes include vaccinia viruses, Venezuelan equine encephalitis virus (VEEV) replicons, adenoviruses, rhabdoviruses vesicular stomatitis virus (VSV) and rabies virus (RABV), and human parainfluenza virus type 3 (HPIV3).

2.3.1 Recombinant Vaccinia Viruses

Vaccinia virus has been the most extensively studied live recombinant vaccine vector [reviewed in Jacobs et al. (2009)]. While recombinant vaccinia viruses have shown utility as vaccine vectors against a number of infectious agents, there have been very few studies which have evaluated this platform against filovirus infection. When this platform was evaluated in the cynomolgus macaque ZEBOV model, the recombinant vaccinia viruses expressing the ZEBOV GP were unable to prolong survival or protect cynomolgus monkeys from lethal ZEBOV infection (Geisbert et al. 2002) (Table 2.5).

2.3.2 Venezuelan Equine Encephalitis Virus Replicons

Alphaviruses have a broad host range and replicate in multiple vertebrate and invertebrate cells. The alphavirus genome contains a single-stranded, positive-sense RNA divided into two open reading frames, one encoding the nonstructural proteins responsible for transcription and replication and the second encoding the structural proteins, which are responsible for encapsidating the viral RNA and final assembly into enveloped virions. Alphaviruses can be employed as vaccine vectors by cloning the gene of interest in place of the alphavirus structural genes. These vectors are commonly called “replicons” and have the ability to replicate but do not make virus particles in the absence of the alphaviral structural proteins. Therefore, alphavirus replicons are single-cycle, non-replicating vectors that cannot spread from cell to cell. Three different expression vectors have been constructed based on alphavirus replicons, including VEEV and Semliki Forest and Sindbis viruses (Rayner et al. 2002; Schlesinger 2001; Lundstrom 2003).

VEEV replicons expressing MARV-Musoke strain (MARV-Musoke) GP either alone or in combination with NP were used to vaccinate cynomolgus macaques against MARV (Hevey et al. 1998) (Table 2.1). The experiment consisted of a regimen of three VEEV replicon injections 28 days apart followed by a high-dose i.m. MARV challenge 35 days after the final immunization. Animals vaccinated with either combination were completely protected against a homologous MARV challenge. NP alone protected against death but did not prevent 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) (Hevey et al. 2001a), which raises the question about the degree of cross-protection of candidate vaccines for the diverse strains of MARV using vaccines expressing

the GP and/or NP proteins. For EBOV, results in NHPs have been inconsistent. Vaccination of cynomolgus monkeys with multiple injections of VEEV replicons expressing either ZEBOV GP, NP, or both GP and NP at doses in the 10^7 pfu range failed to protect any animals from a lethal i.m. ZEBOV challenge (Geisbert et al. 2002) (Table 2.5). Subsequent studies have employed higher doses of VEEV replicons (Table 2.5). Specifically, it was recently shown that a single injection in cynomolgus macaques with blend of 10^{10} VEEV replicons expressing the ZEBOV GP and SEBOV GP was able to protect animals from high-dose (1,000 pfu) i.m. challenge with ZEBOV or SEBOV (Herbert et al. 2013). However, the same test conditions do not appear to afford complete protection against SEBOV if the challenge virus is administered by the aerosol route even when the challenge virus dose is reduced 10-fold (Herbert et al. 2013). In this study, a single injection of 10^{10} VEEV replicons expressing the ZEBOV GP and SEBOV GP was unable to protect any cynomolgus monkeys against an aerosol exposure of 100 pfu of SEBOV. Altering the vaccination regimen to two injections was able to provide protection of macaques against death but not clinical illness.

Currently, the VEEV replicon system faces a number of challenges as an ideal vaccine candidate against filovirus infection due to the inconsistency in studies against ZEBOV and inability to provide cross-protection between strains of MARV, along with protection against homologous MARV requiring a series of three injections over 17 weeks. It does appear that increasing the vaccine dose in the EBOV studies improves protection. However, even with the low dose of vaccine used in the MARV studies (10^7 pfu), the NHPs developed VEEV-neutralizing antibodies after two injections (Hevey et al. 2001b) which raises doubts about the possibility of reusing this system even if the problem of cross-protection against diverse MARV strains can be solved.

2.3.3 *Adenoviruses*

Adenoviruses are 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. Though there have been setbacks, including the death of a patient in 1999 from adverse effects associated with the administration of adenovirus vectors (Lehrman 1999), the interest in their use as a vaccine has remained high, and efforts have focused on developing vectors that have low or no immunogenic toxicities.

Replication-defective adenoviruses, such as the recombinant adenovirus serotype 5 (rAd5), are the most commonly used platform (Hitt and Gauldie 2000). The common feature of all replication-defective rAd vectors is deletion of the viral E1 region that is essential for the regulation of adenovirus transcription and viral replication. Additionally, the E3 region, which is not essential for production of the rAd vectors, is often deleted. The E4 region can also be deleted to increase

capacity for gene inserts and to reduce host responses *in vivo*; however, it must be provided *in trans* for production of recombinant virus (Hitt and Gaudie 2000).

In NHPs, the rAd5 platform has shown remarkable success for filoviral HFs (Sullivan et al. 2000, 2003, 2006, 2011; Swenson et al. 2008a; Pratt et al. 2010; Geisbert et al. 2010a; Richardson et al. 2013) (Tables 2.1, 2.2 and 2.3). Notably, a single i.m. injection with a rAd5-based vaccine expressing MARV-Angola GP resulted in complete protection with no signs of clinical illness in cynomolgus macaques after a high-dose (1,000 pfu) i.m. challenge with MARV-Angola 28 days later (Geisbert et al. 2010a). Additionally four NHPs who received the rAd5 MARV-Angola GP vaccine after three injections of MARV-Angola GP DNA in a prime-boost strategy were also completely protected; however, the failure of the DNA vaccine alone to protect against clinical illness suggests that rAd5 MARV was the key component (Geisbert et al. 2010a).

Sullivan and colleagues were the first to successfully protect NHPs from ZEBOV HF using a prime-boost strategy (Sullivan et al. 2000); cynomolgus monkeys were vaccinated three times with DNA expressing the GPs of ZEBOV, SEBOV, and ICEBOV and the NP of ZEBOV with a booster vaccination of a rAd5 vector expressing the ZEBOV GP 3 months later. All four vaccinated animals survived challenge after week 32 of the vaccination regimen when exposed to a low dose (6 pfu) of ZEBOV. The data for this study revealed that humoral immunity and T memory helper cells were strongly associated with protection; while cell-mediated immunity was important, it was not an absolute requirement for protection (Sullivan et al. 2000). Whether the DNA component of this regimen is absolutely needed is not clear, since there have been no reports 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 (1,000 pfu) i.m. challenge with homologous ZEBOV 28 days later (Sullivan et al. 2003). A DNA prime rAd5 strategy was also employed to demonstrate vaccine efficacy against the most recently discovered species of EBOV, BEBOV (Hensley et al. 2010). In brief, NHPs were initially vaccinated with DNA expressing ZEBOV GP and SEBOV GP and then boosted at weeks 4, 8, and 14. A little over a year later (week 53), animals were boosted with a rAd5-based vaccine expressing the ZEBOV GP. The animals were then challenged 7 weeks after the rAd5 boost with BEBOV. All four specifically vaccinated macaques survived the challenge, and only one animal showed evidence of clinical illness from the BEBOV exposure. While the study showed protection against BEBOV, the regimen requiring five injections over the period of nearly a year and half is not very practical in either natural or biodefense settings.

While filovirus transmission is not thought to be a major route of infection in nature, the inhalation route is among the most likely portals of entry in the setting of a bioterrorist event. Studies have shown 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, while there was only partial protection against an aerosol SEBOV challenge (Pratt et al. 2010). However,

increasing the vaccination regimen to two injections over 99 days completely protected against a SEBOV aerosol challenge (Pratt et al. 2010).

Additionally, a two-injection filovirus vaccine platform 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) (Swenson et al. 2008a). In this study, two groups of cynomolgus monkeys were given an initial i.m. injection of this vaccine and then revaccinated 63 days later. The first group was challenged with MARV-Musoke 42 days after the second vaccination and was protected from lethal disease; this group was then subsequently back-challenged 72 days later with SEBOV. The second group was initially challenged with ZEBOV 43 days after the second vaccination with each animal surviving challenge and then back-challenged 69 days later with MARV-Ci67. All animals in these studies survived the back challenges as well. This platform using the same vaccination strategy has also showed protection against an initial SEBOV challenge (Pratt et al. 2010).

Based on the success of the rAd5 filovirus vaccine platform in NHPs, a phase I clinical trial was conducted using a rAd5 vaccine encoding the ZEBOV and SEBOV GPs. The study consisting of 31 volunteers showed that the vaccine was safe and that subjects developed antigen-specific cellular and humoral immune responses (Ledgerwood et al. 2010). While this study is encouraging, the high prevalence of preexisting immunity to adenoviruses in the human population may substantially limit the immunogenicity and clinical utility of the rAd5-based vaccines. 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 (Schulick et al. 1997; Piedra et al. 1998). Additionally, Merck 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 (Cohen 2007; Sekaly 2008).

Initial attempts to improve adenovirus-based vaccines against filoviruses focused on employing different adenoviruses with lower seroprevalence. However, these initial studies were not very successful (Geisbert et al. 2011). Vaccination of cynomolgus monkeys with recombinant adenovirus serotype 35 (rAd35) expressing the ZEBOV GP failed to completely protect animals against a lethal ZEBOV challenge. Similarly, vaccination of cynomolgus macaques with either recombinant adenovirus serotype 26 (rAd26) 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. A strategy to prime with rAd26 vectors expressing ZEBOV GP and boost with rAd35 vectors expressing ZEBOV GP was able to protect NHPs against a lethal ZEBOV challenge. However, to date, rAd5 is the only adenovirus serotype capable of inducing a protective response against EBOV as a single-injection vaccine. While the data associated with the filovirus rAd5 vaccine platform is impressive, a study revealed that when macaques are pre-immunized against Ad5 and then vaccinated with the rAd5 vaccine expressing the ZEBOV GP,

the NHPs are not protected against disease or death after challenge with ZEBOV (Geisbert et al. 2011) (Table 2.3).

The most recent studies to improve rAd5-based filovirus vaccines have utilized an additional boost with an adenovirus vector expressing interferon (IFN)- α as well as changing the vaccination route (Richardson et al. 2013) (Table 2.3). While it appears that incorporation of a boost vaccination with the adenovirus vector expressing IFN- α did not have any benefit regarding overcoming preexisting immunity, the administration of the rAd5-based ZEBOV GP vaccine by a combined intranasal and intratracheal route did improve survival of NHPs against homologous ZEBOV challenge when compared with vaccination by i.m. injection.

2.3.4 *Rhabdovirus-Based Vaccines*

2.3.4.1 *Vesicular Stomatitis Virus*

Over the last decade, Rose and colleagues have pioneered the use of VSV, the prototypic member of the *Rhabdoviridae* family, as an expression and vaccine vector (Roberts et al. 1999, 2000, 2001). VSV is very suitable as a vaccine expression vector, as it grows to high titer ($>10^9$ pfu/ml) in vitro, can be propagated in almost all mammalian cells, can induce strong humoral as well as cellular responses in vivo, and has the capacity to elicit both mucosal and systemic immunity (Rose et al. 2001; Zinkernagel et al. 1978a, b; Fehr et al. 1996). Furthermore, preexisting immunity to VSV is rare, and infection is not typically associated with serious disease, although VSV-associated encephalitis has been reported (Reif et al. 1987; Gaidamovich et al. 1966; Quiroz et al. 1988).

A recombinant VSV (rVSV)-based system has proven to be among the most successful vaccine platforms for MARV to date and has been proven equally effective against EBOV (Tables 2.1 and 2.4). A single i.m. vaccination of cynomolgus monkeys with a rVSV-MARV-Musoke GP vector elicited complete protection against a high-dose (1,000 pfu) i.m. challenge of homologous MARV given 28 days later (Jones et al. 2005). These animals were also protected when rechallenged 113 days later. Additionally, the MARV-Musoke vaccine proved protective 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 (Daddario-DiCaprio et al. 2006a). Studies have also shown 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 (Geisbert et al. 2008a).

For EBOV, a single i.m. vaccination of cynomolgus monkeys with a rVSV vector expressing only ZEBOV GP also elicited complete protection against a high-dose (1,000 pfu) i.m. challenge of homologous ZEBOV given 28 days later (Jones et al. 2005). However, cross-protection against another species of EBOV,

SEBOV, was not achieved as SEBOV challenge of the survivors resulted in fatal disease (Jones et al. 2005). A single i.m. vaccination of cynomolgus monkeys with rVSV-ZEBOV-GP was also able to completely protect animals against a homologous aerosol challenge of ZEBOV given 28 days later (Geisbert et al. 2008a). Importantly, protection can be conferred by these vaccines via various routes. Immunization of NHPs with the rVSV-ZEBOV-GP vector by either the oral or intranasal route resulted in complete protection of all animals against a high-dose (1,000 pfu) i.m. homologous ZEBOV challenge (Qiu et al. 2009). Recently, the mechanism of rVSV-ZEBOV-GP protection from lethal challenge with ZEBOV was evaluated, and results suggested that antibodies are necessary and correlate with protection of cynomolgus macaques (Marzi et al. 2013).

The ideal filovirus vaccine should be a single-injection vaccine that can protect primates against the various species and/or strains of EBOV and MARV. This is important because endemic areas of filoviruses overlap and since the specific strain or species of filovirus may not be immediately known in the case of a biological weapon attack. With this goal in mind, a study was conducted where cynomolgus monkeys were vaccinated with a multivalent vaccine consisting of equal parts of the rVSV-filovirus-GP vaccine vector for MARV, EBOV, and SEBOV (Geisbert et al. 2009). After 28 days the 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 the utility this platform could have as a single-injection multivalent vaccine.

The BEBOV outbreak in 2007 offered a new challenge to develop a strategy to protect against an emerging species of EBOV using existing vaccines that were available at the time of the outbreak. This strategy was tested in cynomolgus macaques against heterologous challenge with BEBOV. The NHPs in this study were vaccinated with rVSV-ZEBOV-GP or rVSV-ICEBOV-GP separately and challenged with BEBOV 28 days after vaccination. While the rVSV-ICEBOV-GP vector did not provide any additional protection when compared to mock-vaccinated control NHPs in the study (33 % survival), the rVSV-ZEBOV-GP vaccine protected 75 % of animals against lethal infection (Falzarano et al. 2011). Recently, the utility of combining rVSV-SEBOV-GP and rVSV-ZEBOV-GP vectors using either a single-injection blended vaccination approach or in a prime-boost regimen against heterologous BEBOV challenge in cynomolgus macaques was evaluated (Mire et al. 2013). Furthermore, the ability of a single injection of a newly developed homologous rVSV-BEBOV-GP vaccine vector to provide protection against homologous BEBOV challenge was assessed in this study. The rVSV-BEBOV-GP vector protected against homologous challenge with BEBOV. The prime-boost strategy with the rVSV-SEBOV-GP (prime) and rVSV-ZEBOV-GP (boost 14 days post rVSV-SEBOV-GP vaccination) vectors, which were available at the time BEBOV emerged, was capable of providing cross-protection against BEBOV challenge 35 days after prime vaccination. These results were promising and showed that a condensed, prime-boost vaccine regimen of available heterologous rVSV-filovirus-GP vaccines could be considered as a paradigm for controlling newly emerging EBOV species.

In addition to its efficacy as a preventive vaccine, the rVSV vaccine platform has also been used as a postexposure treatment for filovirus infections (Table 2.6). Remarkably, treatment of rhesus macaques with rVSV-MARV-GP shortly after a homologous high-dose MARV challenge resulted in complete protection of all animals from clinical illness and death (Daddario-DiCaprio et al. 2006b). Subsequent studies demonstrated that the rVSV-filovirus-GP vectors for ZEBOV and SEBOV protected 50 and 100 % of rhesus macaques, respectively, when administered as postexposure prophylaxis after high-dose homologous virus challenge (Feldmann et al. 2007; Geisbert et al. 2008b). The rVSV-filovirus-GP vectors were administered 20–30 min after filovirus challenge in these studies. A major question is how long after virus exposure can the rVSV-filovirus-GP vectors be effective? To address this question, rhesus macaques were treated with rVSV-MARV-GP 24 h post-homologous MARV challenge which 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 (Geisbert et al. 2010b).

Replication-competent vaccines, including the rVSV platform, are not without concern when it comes to their safety, especially in immunocompromised persons. However, initial results of various rVSV vectors in NHPs have been promising as no toxicity was seen in rhesus macaques following intranasal inoculation with wild-type VSV, rVSV wild-type, and two rVSV-HIV vectors, although neurovirulence was noted in one of four animals after direct intrathalamic inoculation of rVSV (Johnson et al. 2007). To date, no toxicity has been seen in over 100 NHPs given rVSV-MARV or rVSV-EBOV (Jones et al. 2005; Daddario-DiCaprio et al. 2006a; Geisbert et al. 2008a, 2009; Qiu et al. 2009; Falzarano et al. 2011; Marzi et al. 2013; Mire et al. 2013). Furthermore, there has been no significant vaccine vector shedding detected in these experiments despite immunization doses of up to 10^7 pfu (Jones et al. 2005; Daddario-DiCaprio et al. 2006a; Geisbert et al. 2008a, 2009, 2008c; Qiu et al. 2009; Falzarano et al. 2011; Marzi et al. 2013; Mire et al. 2013) which suggests, along with the natural low transmissibility of VSV (Tesh et al. 1975; Hanson 1952), 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, NOD-SCID mice (Jones et al. 2007) and SHIV-infected rhesus monkeys (Geisbert et al. 2008c), along with neurovirulence testing of the rVSV-ZEBOV-GP and rVSV-MARV-GP vaccines in cynomolgus macaques by intrathalamic injection (Mire et al. 2012). No evidence of overt illness or neurovirulence was noted in any of the animals. In addition, the rVSV-ZEBOV-GP vector was recently used to treat a laboratory worker after a recent laboratory accident (Gunther et al. 2011). 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. 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 (Slate et al. 2005) with no evidence of evolution to more pathogenic forms.

2.3.4.2 Rabies Virus

Recently, a RABV bivalent filovirus vaccine platform (RABV/ZEBOV) was tested in NHPs (Blaney et al. 2013) (Table 2.5). Four groups of rhesus macaques were used in this study. Group 1 consisted of three control animals receiving a single injection of the RABV vaccine (BNSP333); group 2 consisted of four animals that received a single injection of the bivalent RABV/ZEBOV vaccine vector (BNSP333-GP); group 3 had four animals that received a single injection of the replication-restricted RABV vector expressing the ZEBOV GP (BNSP Δ G-GP); and group 4 consisted of four animals that received two doses (a prime and boost 28 days later) with a beta-propiolactone inactivated version of the BNSP333-GP vector. The vaccinated NHPs were challenged 70 days after vaccination and followed for up to 28 days post challenge.

The study showed that all animals in groups 2 and 3 were able to generate a humoral immune response to the ZEBOV GP immunogen with the humoral immune response being the major response when compared to the cellular response during the vaccination phase of the study. ZEBOV viremia was detected in all groups to some extent with all animals in group 1, one of four animal in groups 2 and 3, and three of four animals in group 4 all having viremia at day 6 post challenge. Each control in group 1 succumbed to ZEBOV challenge with 50 % succumbing for groups 3 and 4, whereas all the animals in group 2 survived the lethal challenge. These results were interesting considering all animals in groups 2–4 had generated IgG antibodies against the ZEBOV GP with no differences in the avidity of the antibodies between the groups. When the IgG response was further analyzed, it was found that the IgG1 response was important for protection using this vaccine vector suggesting that there is an antibody-dependent cellular cytotoxicity and complement activation mechanism for protection in the NHPs. While these results are promising, this system has yet to be tested for safety in NHPs although neurovirulence studies in mice were encouraging (Papaneri et al. 2012). In addition, this study utilized rhesus macaques for testing in a preventive vaccination setting, and further studies in the more robust cynomolgus model will be needed for better comparison with other filovirus vaccines.

2.3.5 Human Parainfluenza Virus Type 3

Human parainfluenza virus type 3 (HPIV3), a member of the family *Paramyxoviridae*, is a common pediatric respiratory pathogen. Live-attenuated vectors based on HPIV3 are actively being investigated as vaccines for HPIV3

and other pediatric pathogens (Durbin et al. 2000; Karron et al. 2003). Bukreyev and colleagues recently developed HPIV3 a vector expressing the ZEBOV GP and a vector expressing two antigens the ZEBOV GP and NP (Bukreyev et al. 2007) (Table 2.5). Combining an intranasal and intratracheal vaccination in cynomolgus macaques with the ZEBOV GP afforded the best protection against a high-dose (1,000 pfu) intraperitoneal (i.p.) challenge of homologous ZEBOV 28 days post vaccination (Geisbert et al. 2008a). In this study, six of seven HPIV3 ZEBOV GP-vaccinated animals survived challenge, and four of seven animals were protected against clinical illness. When the vaccine regimen using the HPIV3 ZEBOV GP vaccine was expanded to two doses over a 67-day period, the efficacy was improved, with three of three cynomolgus monkeys protected against clinical illness and death.

The HPIV3 system is replication competent which brings up similar safety concerns as the rVSV platform. Additionally, a majority of all adult humans have preexisting immunity to this common childhood pathogen, which presents a potential challenge using this vaccine vector analogous to rAd5. This concern was recently addressed as it was demonstrated that reinfection of NHPs with HPIV3 expressing ZEBOV GP is possible and results in an immune response to ZEBOV GP, indicating that vaccination might be feasible despite preexisting immunity (Bukreyev et al. 2010). Unfortunately, this study did not provide data on protective efficacy which as seen with the RABV/ZEBOV vector antibody response does not always correlate with protection (Blanney et al. 2013).

2.4 Conclusions and Future Directions

While initial progress on developing vaccines against filoviruses was slow, tremendous progress has been made over the last decade. At least four viral-vectored vaccines 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 a postexposure prophylaxis. This progress was so encouraging that efforts were made to move candidate filovirus vaccines into clinical trials. However, there have been four recent events that have caused concern and have dampened enthusiasm for rapid movement toward vaccines suitable for human use. These include: (1) the recent awareness that the Angola strain of MARV is more pathogenic in primates than strains that most vaccines had been tested against; (2) the discovery of the new BEBOV species of EBOV in 2007; (3) studies suggesting that aerosol exposure may be more difficult to protect against than intramuscular injection; and (4) concerns regarding most vaccine studies in NHPs being tested against an attenuated cell culture variant of ZEBOV.

Studies have shown that the Angola strain of MARV causes a higher case fatality rate in humans and more rapid disease course in NHPs than other MARV strains. Early vaccine efficacy studies focused on the Musoke strain of MARV which was derived from a nonfatal human case and causes a more protracted disease in NHPs.

While rAd5- and rVSV-based vaccines have recently been shown to confer protection against this MARV strain, other vaccines have yet to be assessed. A greater concern than MARV-Angola is the newest species of EBOV, BEBOV. Three studies have evaluated vaccines in NHPs against BEBOV. One study using a rVSV-based BEBOV GP single-injection vaccine demonstrated complete protection of NHPs against disease from a homologous BEBOV challenge. However, multivalent vaccines comprised of ZEBOV and/or SEBOV antigens while protecting animals against death were unable to protect all animals from clinical illness. Moreover, no single-injection vaccine containing ZEBOV and/or SEBOV antigens has been able to completely protect NHPs from lethal BEBOV challenge. This complicates vaccine development as single-injection protection may require incorporation of BEBOV antigens further adding to the number of components in a filovirus vaccine.

An ideal filovirus vaccine would protect in a natural setting which includes contact or parenteral routes of exposure and in a biodefense scenario which would likely involve aerosol exposure. While one study using rVSV-based vaccines demonstrated protection of NHPs from homologous ZEBOV or MARV challenge, another study suggests that protection against aerosol exposure may present additional challenges. Specifically, it was recently shown that a VEEV replicon-based vaccine was unable to protect any NHPs against homologous SEBOV infection when administered as a single-injection vaccine. A prime-boost strategy with the same vaccine was able to protect all animals against homologous SEBOV challenge; however, all animals experienced more than mild clinical disease.

Perhaps the most significant hurdle that has recently been encountered in advancing filovirus vaccines for human use involves the recent discovery that ZEBOV seed stocks have acquired mutations in the GP gene by cell culture passage (Volchkova et al. 2011; Kugelman et al. 2012) and these viruses appear to be less pathogenic in NHPs (Geisbert 2013). Specifically, for EBOVs, the GP gene expresses three different products (the spike GP1,2, the soluble (s)GP, and small soluble (ss)GP) by alternating the use of three overlapping reading frames (ORFs). The ratio of expression of these three proteins is controlled by a stretch of seven (7) consecutive template uridines (7U) known as the RNA editing site. sGP is the primary expression product of the GP gene. However, it has recently been shown that cell culture passage has a tendency to cause the insertion of an additional U residue in the viral genome at the GP editing site (8U) (Volchkova et al. 2011; Kugelman et al. 2012). One of these studies also showed that the majority of ZEBOV seed stocks used to evaluate candidate filovirus vaccines contain higher proportions of the 8U mutation than the wild-type 7U sequence (Kugelman et al. 2012). This mutation may have a very significant impact on virulence as it has a direct effect on the amount of sGP produced by infected cells. For wild-type EBOVs with the 7U phenotype, 80 % of the product of the GP gene is sGP. Cell culture-adapted EBOVs with higher 8U content and lower 7U content will thus produce proportionally lower amounts of sGP. While the function of sGP is unknown, it has speculated to act as a decoy and plays a role in subverting the host immune response (Volchikov et al. 1998; Ito et al. 2001; Mohan et al. 2012).

Interestingly, recent studies have shown that ZEBOV seed stocks with high proportions of 7U at the GP editing site cause a more rapid disease course in cynomolgus macaques than ZEBOV stocks with high proportions of 8U at the GP editing site (C.E. Mire, T.W. Geisbert, unpublished observations). More importantly, recent testing of several candidate vaccines that previously were shown to provide complete protection of NHPs against 8U ZEBOV infection indicated that only one of these vaccines, rVSV, was able to provide complete protection of macaques against a predominantly 7U ZEBOV seed stock (Geisbert 2013). Clearly, more work needs to be conducted to better define the impact of the 8U mutation on virulence and the ability of candidate vaccines to protect against the wild-type 7U EBOV seed stocks.

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 “animal rule” enacted by the US FDA in 2002 [reviewed in Roberts and McCune (2008)], which established requirements for the evidence needed to demonstrate effectiveness of new drugs and biological products when human efficacy studies are not ethical or feasible. This rule would most likely be enacted for filoviral HF drugs and vaccines. This rule states that a product can be licensed based on evidence of effectiveness derived from studies in well-characterized animal models and the usual demonstration of biological activity and safety in humans. Thus, the validation of NHPs as accurate and reliable models of human filoviral HF has been and will be critical to the final evaluation and testing of candidate vaccines. Ultimately, no vaccine against filovirus infection will be approved for human use until it can protect NHPs from viremia and clinical illness.

The extent to which laboratory animal models corroborate with findings in humans is very important in the characterization of filovirus pathogenesis. 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 the sporadic outbreaks in Central Africa (Bausch et al. 2008). In particular, a deeper understanding of the correlates of immunity in both humans and animal models of filoviral HF will be paramount to achieving this goal.

Lastly, if FDA approval of a filovirus vaccine should occur, there are still questions about economic incentives for pharmaceutical companies to produce a MARV or EBOV countermeasure. The areas most affected by outbreaks of filoviruses are generally in poor countries, so economic incentives will have to come from other nations or private foundations. However, the concerns of industrialized countries when it comes to protecting the military and others considered susceptible to use of filoviruses as bioweapons are the most likely driving force. The responsibility will then become the international community's to ensure that these vaccines are available to the persons in the most need in endemic areas where pathogenic filoviruses are found in nature.

References

- Baize S, Leroy EM, Georges-Courbot MC et al (1999) Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. *Nat Med* 5:423–426
- Barrette RW, Metwally SA, Rowland JM et al (2009) Discovery of swine as a host for the Reston ebolavirus. *Science* 325:204–206
- Bausch DG, Sprecher AG, Jeffs B, Boumandouki P (2008) Treatment of Marburg and Ebola hemorrhagic fevers: a strategy for testing new drugs and vaccines under outbreak conditions. *Antiviral Res* 78:150–161
- Bechtelsheimer H, Korb G, Gedigk P (1971) Marburg virus hepatitis. In: Martini GA, Siebert R (eds) *Marburg virus disease*. Springer, New York, NY, pp 62–67
- Blaney JE, Marzi A, Willet M et al (2013) Antibody quality and protection from lethal Ebola virus challenge in nonhuman primates immunized with rabies virus based bivalent vaccine. *PLoS Pathog* 9:e1003389
- Bradfute SB, Braun DR, Shamblin JD et al (2007) Lymphocyte death in a mouse model of Ebola virus infection. *J Infect Dis* 196(Suppl 2):S296–S304
- Bray M, Davis K, Geisbert T, Schmaljohn C, Huggins J (1998) A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. *J Infect Dis* 178:651–661
- Bray M, Hatfill S, Hensley L, Huggins JW (2001) Haematological, biochemical and coagulation changes in mice, guinea-pigs and monkeys infected with a mouse-adapted variant of Ebola Zaire virus. *J Comp Pathol* 125:243–253
- Bukreyev A, Rollin PE, Tate MK et al (2007) Successful topical respiratory tract immunization of primates against Ebola virus. *J Virol* 81:6379–6388
- Bukreyev AA, Dinapoli JM, Murphy BR, Collins PL (2010) Mucosal parainfluenza virus-vectored vaccine against Ebola virus replicates in the respiratory tract of vector-immune monkeys and is immunogenic. *Virology* 399:290–298
- Cohen J (2007) AIDS research. Did Merck's failed HIV vaccine cause harm? *Science* 318:1048–1049
- Connolly BM, Steele KE, Davis KJ et al (1999) Pathogenesis of experimental Ebola virus infection in guinea pigs. *J Infect Dis* 179(Suppl 1):S203–S217
- Daddario-DiCaprio KM, Geisbert TW, Geisbert JB et al (2006a) Cross-protection against Marburg virus strains using a live, attenuated recombinant vaccine. *J Virol* 80:9659–9666
- Daddario-DiCaprio KM, Geisbert TW, Stroher U et al (2006b) Post-exposure protection against Marburg haemorrhagic fever with recombinant vesicular stomatitis virus vectors in non-human primates: an efficacy assessment. *Lancet* 367:1399–1404
- Durbin AP, Skiadopoulos MH, Riggs JM et al (2000) Human parainfluenza virus type 3 (PIV3) expressing hemagglutinin protein of measles virus provides a potential method for immunization against measles virus and PIV3 in early infancy. *J Virol* 74:6821–6831
- Ebihara H, Zivcec M, Gardner D et al (2013) A Syrian golden hamster model recapitulating ebola hemorrhagic fever. *J Infect Dis* 207:306–318
- Falzarano D, Feldmann F, Grolla A et al (2011) Single immunization with a monovalent vesicular stomatitis virus-based vaccine protects nonhuman primates against heterologous challenge with Bundibugyo ebolavirus. *J Infect Dis* 204(Suppl 3):S1082–S1089
- Fehr T, Bachmann MF, Bluethmann H et al (1996) T-independent activation of B cells by vesicular stomatitis virus: no evidence for the need of a second signal. *Cell Immunol* 168:184–192
- Feldmann H, Jones SM, Daddario-Dicaprio KM et al (2007) Effective post-exposure treatment of Ebola infection. *PLoS Pathog* 3:e2
- Feldmann H, Sanchez A, Geisbert TW (2013) Filoviridae: Ebola and Marburg viruses. In: Knipe DM, Howley PM (eds) *Fields virology*, 6th edn. Lippincott Williams & Wilkins, Philadelphia, PA, pp 923–956

- Gaidamovich S, Uvarov VN, Alekseeva AA (1966) Isolation of vesicular stomatitis virus from a patient. *Vopr Virusol* 11:77–80
- Geisbert TW (2013) Progress in the development of vaccines against Ebola and Marburg viruses. In: 11th ASM biodefense and emerging diseases research meeting, Washington, DC, 26 February 2013
- Geisbert TW, Hensley LE, Gibb TR, Steele KE, Jaax NK, Jahrling PB (2000) Apoptosis induced in vitro and in vivo during infection by Ebola and Marburg viruses. *Lab Invest* 80:171–186
- Geisbert TW, Pushko P, Anderson K, Smith J, Davis KJ, Jahrling PB (2002) Evaluation in nonhuman primates of vaccines against Ebola virus. *Emerg Infect Dis* 8:503–507
- Geisbert TW, Daddario-DiCaprio KM, Geisbert JB et al (2008a) Vesicular stomatitis virus-based vaccines protect nonhuman primates against aerosol challenge with Ebola and Marburg viruses. *Vaccine* 26:6894–6900
- Geisbert TW, Daddario-DiCaprio KM, Williams K et al (2008b) Recombinant vesicular stomatitis virus vector mediates post-exposure protection against Sudan Ebola hemorrhagic fever in nonhuman primates. *J Virol* 82:5664–5668
- Geisbert TW, Daddario-DiCaprio KM, Lewis MG et al (2008c) Vesicular stomatitis virus-based Ebola vaccine is well-tolerated and protects immunocompromised nonhuman primates. *PLoS Pathog* 4:e1000225
- Geisbert TW, Geisbert JB, Leung A et al (2009) Single injection vaccine protects nonhuman primates against Marburg virus and three species of Ebola virus. *J Virol* 83:7296–7304
- Geisbert TW, Bailey M, Geisbert JB et al (2010a) Vector choice determines immunogenicity and potency of genetic vaccines against Angola Marburg virus in nonhuman primates. *J Virol* 84:10386–10394
- Geisbert TW, Hensley LE, Geisbert JB et al (2010b) Postexposure treatment of Marburg virus infection. *Emerg Infect Dis* 16:1119–1122
- Geisbert TW, Bailey M, Hensley L et al (2011) Recombinant adenovirus serotypes 26 and 35 vaccine vectors bypass immunity to Ad5 and protect nonhuman primates against Ebola virus challenge. *J Virol* 85:4222–4233
- Gunther S, Feldmann H, Geisbert TW et al (2011) Management of accidental exposure to Ebola virus in the biosafety level 4 laboratory, Hamburg, Germany. *J Infect Dis* 204(Suppl 3):S785–S790
- Hanson RP (1952) The natural history of vesicular stomatitis. *Bacteriol Rev* 16:179–204
- Hensley LE, Mulangu S, Asiedu C et al (2010) Demonstration of cross-protective vaccine immunity against an emerging pathogenic Ebola virus species. *PLoS Pathog* 6:e1000904
- Herbert AS, Kuehne AI, Barth JF et al (2013) Venezuelan equine encephalitis virus replicon particle vaccine protects nonhuman primates from intramuscular and aerosol challenge with ebolavirus. *J Virol* 87:4952–4964
- Hevey M, Negley D, Pushko P, Smith J, Schmaljohn A (1998) Marburg virus vaccines based upon alphavirus replicons protect guinea pigs and nonhuman primates. *Virology* 251:28–37
- Hevey M, Negley D, Staley A, Schmaljohn A (2001) Determination of vaccine components required for protecting cynomolgus macaques against genotypically divergent isolates of Marburg virus. In: 20th Annual Meeting of the American society for virology, Madison, WI, USA. Abstract No. W36-4
- Hevey M, Negley D, VanderZanden L et al (2001b) Marburg virus vaccines: comparing classical and new approaches. *Vaccine* 20:586–593
- Hitt MM, Gauldie J (2000) Gene vectors for cytokine expression in vivo. *Curr Pharm Des* 6:613–632
- Ito H, Watanabe S, Takada A, Kawaoka Y (2001) Ebola virus glycoprotein: proteolytic processing, acylation, cell tropism, and detection of neutralizing antibodies. *J Virol* 75:1576–1580
- Jacobs BL, Langland JO, Kibler KV et al (2009) Vaccinia virus vaccines: past, present and future. *Antiviral Res* 84:1–13

- Johnson JE, Nasar F, Coleman JW et al (2007) Neurovirulence properties of recombinant vesicular stomatitis virus vectors in non-human primates. *Virology* 360:36–49
- Jones SM, Feldmann H, Stroher U et al (2005) Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nat Med* 11:786–790
- Jones SM, Stroher U, Fernando L et al (2007) Assessment of a vesicular stomatitis virus-based vaccine by use of the mouse model of Ebola virus hemorrhagic fever. *J Infect Dis* 196(Suppl 2):S404–S412
- Karron RA, Belshe RB, Wright PF et al (2003) A live human parainfluenza type 3 virus vaccine is attenuated and immunogenic in young infants. *Pediatr Infect Dis J* 22:394–405
- Kugelman JR, Lee MS, Rossi CA et al (2012) Ebola virus genome plasticity as a marker of its passing history: a comparison of in vitro passaging to non-human primate infection. *PLoS One* 7:e50316
- Le Guenno B, Formenty P, Wyers M, Gounon P, Walker F, Boesch C (1995) Isolation and partial characterisation of a new strain of Ebola virus. *Lancet* 345:1271–1274
- Ledgerwood JE, Costner P, Desai N et al (2010) A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. *Vaccine* 29:304–313
- Lehrman S (1999) Virus treatment questioned after gene therapy death. *Nature* 401:517–518
- Lundstrom K (2003) Alphavirus vectors for vaccine production and gene therapy. *Expert Rev Vaccines* 2:447–459
- Marzi A, Engelmann F, Feldmann F et al (2013) Antibodies are necessary for rVSV/ZEBOV-GP-mediated protection against lethal Ebola virus challenge in nonhuman primates. *Proc Natl Acad Sci U S A* 110:1893–1898
- Mire CE, Miller AD, Carville A et al (2012) Recombinant vesicular stomatitis virus vaccine vectors expressing filovirus glycoproteins lack neurovirulence in nonhuman primates. *PLoS Negl Trop Dis* 6:e1567
- Mire CE, Marzi A, Geisbert JB et al (2013) Vesicular stomatitis virus-based vaccines protect nonhuman primates against Bundibugyo Ebola virus. *PLoS Negl Trop Dis* 7(12):e2600
- Mohan GS, Li W, Ye L, Compans RW, Yang C (2012) Antigenic subversion: a novel mechanism of host immune evasion by Ebola virus. *PLoS Pathog* 8:e1003065
- Papaneri AB, Wirblich C, Cann JA et al (2012) A replication-deficient rabies virus vaccine expressing Ebola virus glycoprotein is highly attenuated for neurovirulence. *Virology* 434:18–26
- Piedra PA, Poveda GA, Ramsey B, McCoy K, Hiatt PW (1998) Incidence and prevalence of neutralizing antibodies to the common adenoviruses in children with cystic fibrosis: implication for gene therapy with adenovirus vectors. *Pediatrics* 101:1013–1019
- Pratt WD, Wang D, Nichols DK et al (2010) Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector. *Clin Vaccine Immunol* 17:572–581
- Qiu X, Fernando L, Alimonti JB et al (2009) Mucosal immunization of cynomolgus macaques with the VSVDeltaG/ZEBOV GP vaccine stimulates strong Ebola GP-specific immune responses. *PLoS One* 4:e5447
- Quiroz E, Moreno N, Peralta PH, Tesh RB (1988) A human case of encephalitis associated with vesicular stomatitis virus (Indiana serotype) infection. *Am J Trop Med Hyg* 39:312–314
- Rayner JO, Dryga SA, Kamrud KI (2002) Alphavirus vectors and vaccination. *Rev Med Virol* 12:279–296
- Reif JS, Webb PA, Monath TP et al (1987) Epizootic vesicular stomatitis in Colorado, 1982: infection in occupational risk groups. *Am J Trop Med Hyg* 36:177–182
- Richardson JS, Pillet S, Bello AJ, Kobinger GP (2013) Airway delivery of an adenovirus-based Ebola virus vaccine bypasses existing immunity to homologous adenovirus in nonhuman primates. *J Virol* 87:3668–3677
- Roberts R, McCune SK (2008) Animal studies in the development of medical countermeasures. *Clin Pharmacol Ther* 83:918–920

- Roberts A, Buonocore L, Price R, Forman J, Rose JK (1999) Attenuated vesicular stomatitis viruses as vaccine vectors. *J Virol* 73:3723–3732
- Rose NF, Roberts A, Buonocore L, Rose JK (2000) Glycoprotein exchange vectors based on vesicular stomatitis virus allow effective boosting and generation of neutralizing antibodies to a primary isolate of human immunodeficiency virus type 1. *J Virol* 74:10903–10910
- Rose NF, Marx PA, Luckay A et al (2001) An effective AIDS vaccine based on live attenuated vesicular stomatitis virus recombinants. *Cell* 106:539–549
- Ryabchikova E, Kolesnikova L, Smolina M et al (1996) Ebola virus infection in guinea pigs: presumable role of granulomatous inflammation in pathogenesis. *Arch Virol* 141:909–921
- Schlesinger S (2001) Alphavirus vectors: development and potential therapeutic applications. *Expert Opin Biol Ther* 1:177–191
- Schulick AH, Vassalli G, Dunn PF et al (1997) Established immunity precludes adenovirus-mediated gene transfer in rat carotid arteries. Potential for immunosuppression and vector engineering to overcome barriers of immunity. *J Clin Invest* 99:209–219
- Sekaly RP (2008) The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development? *J Exp Med* 205:7–12
- Slate D, Rupprecht CE, Rooney JA, Donovan D, Lein DH, Chipman RB (2005) Status of oral rabies vaccination in wild carnivores in the United States. *Virus Res* 111:68–76
- Sullivan NJ, Sanchez A, Rollin PE, Yang ZY, Nabel GJ (2000) Development of a preventive vaccine for Ebola virus infection in primates. *Nature* 408:605–609
- Sullivan NJ, Geisbert TW, Geisbert JB et al (2003) Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. *Nature* 424:681–684
- Sullivan NJ, Geisbert TW, Geisbert JB et al (2006) Immune protection of nonhuman primates against Ebola virus with single low-dose adenovirus vectors encoding modified GPs. *PLoS Med* 3:e177
- Sullivan NJ, Hensley L, Asiedu C et al (2011) CD8(+) cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. *Nat Med* 17:1128–1131
- Swenson DL, Wang D, Luo M et al (2008a) Complete protection of nonhuman primates against multi-strain Ebola and Marburg virus infections. *Clin Vaccine Immunol* 15:460–467
- Swenson DL, Warfield KL, Larsen T, Alves DA, Coberley SS, Bavari S (2008b) Monovalent virus-like particle vaccine protects guinea pigs and nonhuman primates against infection with multiple Marburg viruses. *Expert Rev Vaccines* 7:417–429
- Tesh RB, Johnson KM (1975) Vesicular stomatitis. In: Hubbert WT, Mccolloch WF, Schnurrenberger PR (eds) *Diseases transmitted from animals to man*. CC Thomas, Springfield, pp 897–910
- Volchkov VE, Volchkova VA, Slenczka W, Klenk HD, Feldmann H (1998) Release of viral glycoproteins during Ebola virus infection. *Virology* 245:110–119
- Volchkova VA, Dolnik O, Martinez MJ, Reynard O, Volchkov VE (2011) Genomic RNA editing and its impact on Ebola virus adaptation during serial passages in cell culture and infection of guinea pigs. *J Infect Dis* 204(Suppl 3):S941–S946
- Warfield KL, Swenson DL, Olinger GG, Kalina WV, Aman MJ, Bavari S (2007) Ebola virus-like particle-based vaccine protects nonhuman primates against lethal Ebola virus challenge. *J Infect Dis* 196(Suppl 2):S430–S437
- Warfield KL, Bradfute SB, Wells J et al (2009) Development and characterization of a mouse model for Marburg hemorrhagic fever. *J Virol* 83:6404–6415
- Zinkernagel RM, Althage A, Holland J (1978a) Target antigens for H-2-restricted vesicular stomatitis virus-specific cytotoxic T cells. *J Immunol* 121:744–748
- Zinkernagel RM, Adler B, Holland JJ (1978b) Cell-mediated immunity to vesicular stomatitis virus infections in mice. *Exp Cell Biol* 46:53–70
- Zlotnik I (1971) Marburg virus disease. The pathology of experimentally infected hamsters. In: Martini GA, Siebert R (eds) *Marburg virus disease*. Springer, New York, NY, pp 129–135

<http://www.springer.com/978-3-7091-1817-7>

Novel Technologies for Vaccine Development

Lukashevich, I.; Shirwan, H. (Eds.)

2014, XIV, 386 p. 35 illus., 33 illus. in color., Hardcover

ISBN: 978-3-7091-1817-7