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Chikungunya Virus Vaccines: Viral Vector–Based Approaches

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In 2013, a major chikungunya virus (CHIKV) epidemic reached the Americas. In the past 2 years, >1.7 million people have been infected. In light of the current epidemic, with millions of people in North and South America at risk, efforts to rapidly develop effective vaccines have increased. Here, we focus on CHIKV vaccines that use viral-vector technologies. This group of vaccine candidates shares an ability to potently induce humoral and cellular immune responses by use of highly attenuated and safe vaccine backbones. So far, well-described vectors such as modified vaccinia virus Ankara, complex adenovirus, vesicular stomatitis virus, alphavirus-based chimeras, and measles vaccine Schwarz strain (MV/Schw) have been described as potential vaccines. We summarize here the recent data on these experimental vaccines, with a focus on the preclinical and clinical activities on the MV/Schw-based candidate, which is the first CHIKV-vectored vaccine that has completed a clinical trial.

Keywords. chikungunya virus; vaccine; viral vectors; measles virus vector; MVA; adenovirus vector; VSV.

Chikungunya virus (CHIKV) is a positive-stranded RNA alphavirus that is transmitted by *Aedes* mosquitoes. Infection is in most cases a symptomatic acute febrile illness. In up to 30% of patients, the virus can cause debilitating arthritis that lasts for months or years [1, 2]. Consequently, CHIKV epidemics present major public health threats because of substantial morbidity, suffering, and loss of economic productivity, especially in low-income countries. In the past decade, a number of research efforts have been launched to develop CHIKV vaccines [3]. However, owing to the low incidence of CHIKV infections after the 2005–2006 La Réunion epidemic, research activities slowed until the first cases of CHIKV infection were reported in December 2013 on the Caribbean island of Martinique. More than 1.7 million people have been infected in <2 years, which has substantially fueled the development efforts for a CHIKV vaccine.

A potential CHIKV vaccine should induce a protective immune response after 1 or 2 immunizations, to enable effective immunization programs in CHIKV-endemic areas, as well as for travelers. In addition, the vaccine has to be easily manufactured at low cost, as low-income countries are disproportionately affected by the disease. Currently, no correlate of protection for CHIKV has been established. However, the general opinion is that the level of CHIKV neutralizing antibodies correlates with the protection against disease. The role of T cells in protection against disease is still under investigation, but CHIKV-specific T-cell activation occurs early in infection and likely plays a role in control of viral infection prior to antibody responses [4]. CHIKV circulates in 3 genotypes: Asian, East/Central/South African (ECSA), and West African. All strains are closely related, with 95.2%–99.8% amino acid similarity [5]. The high similarity allows potential protection against heterologous strains when vaccinated with a single genotype. Thus, a cross-protective CHIKV vaccine can be produced using a single-genetic-lineage vaccine antigen. Current vaccines under development include those containing attenuated CHIKV strains, recombinant protein vaccines (subunit and virus-like particles [VLPs]), formalin-inactivated vaccines, DNA vaccines, and viral-vectored vaccines [3].

VIRAL VECTOR VACCINES

Today, numerous live-attenuated vaccines are approved, and some of them have been used for decades [6]. The excellent efficacy and safety of this class of vaccines are well established and have allowed the further development of vector systems to deliver antigen originating from other pathogens. The use of viral vectors is a potent tool in gene therapy and vaccine development because of their ability to induce both potent humoral and cellular immune responses. Heterologous antigens can be expressed and delivered at the relevant tissues to generate the most efficient pathogen-specific host responses. In addition, the immunogenicity is further enhanced through intrinsic vector motifs that stimulate the innate immunity pathways [7, 8]. Thus, the use of expensive and mostly reactive adjuvants can be omitted. Viral vectors can use the host-cell protein-processing pathways that lead to antigen presentation via major histocompatibility complex class I and consequent cytotoxic T-cell stimulation [9]. In addition, viral vectors can be

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produced in high quantities at relatively low costs, which allows the use of these systems in low-income countries. Despite the many advantages of potential viral-vector vaccines, preexisting antivector immunity is a major obstacle for development pathways [10]. Vector-specific antibodies may impede the induction of immune responses of the heterologous expressed proteins by reducing the dose and time of exposure to the vaccine antigen [11, 12]. Strategies to overcome this possible problem include increasing vector dose [13, 14], using vectors derived from nonhuman pathogens (eg, chimpanzee adenovirus [Ad] and vesicular stomatitis virus [VSV]) [15], or using heterologous prime-boost approaches [16]. Current strategies to develop CHIKV vaccines based on viral-vector technologies are summarized in Table 1.

MEASLES VACCINE VECTOR

The live recombinant measles vaccine vector technology has many advantages. This technology is based on measles virus (MV) vaccine Schwarz strain (MV/Schw), a live-attenuated negative-stranded RNA virus, which is one of the safest and most effective human vaccines. The measles vaccine has been widely used in the past 40 years to prevent MV infection and has dramatically reduced childhood mortality from measles by 95% since its introduction. The vaccine is very safe and induces a lifelong protective immunity after a single immunization [27]. The entire virus life cycle is limited to the cytoplasm, which prevents insertion of foreign genes into the host genome. In addition, vaccine doses can be produced on a large scale and distributed at low cost. Conventional and recombinant measles vaccine strains have been extensively studied in past decades as tools in cancer therapy, owing to their intrinsic ability to target and destroy cancer cells. A number of clinical trials have been conducted, in many cases using very high doses (3-4 logs higher, compared with MV vaccine), further demonstrating the safety of this viral vector system [28]. The MV vector thus appears appropriate for developing a safe and cost-effective CHIKV vaccine to be used in the case of CHIKV emergence.

A reverse genetic system for the rescue of the negative-stranded MV genome was established [29]. Single or multiple heterologous genes can be expressed stably from the recombinant vectors, with a total size of approximately 6 kb [30]. Tangy et al at the Pasteur Institute in Paris introduced the live-attenuated MV/Schw as a vector to express heterologous viral antigens [31]. A complementary DNA (cDNA) plasmid was generated that contains the full-length infectious antigenome of MV/Schw. Additional ATU sites inserted at different measles virus genomic regions allow the introduction of heterologous genes, which are under the control of *cis*-acting promoter elements modeled after those present in the nucleocapsid/phosphoprotein boundary region, allowing a transcriptional stop of the upstream gene, as well as autonomous transcription, capping, and polyadenylation of the transgene [31].

To date, the MV Schwarz vector has proven to be an effective tool to generate recombinant MV clones expressing heterologous viral antigens, including West Nile virus [32], dengue virus [33], human immunodeficiency virus [34, 35], and SARS coronavirus [36]. The recombinant vaccines induced a strong,

Table 1. Vectored Chikungunya Virus (CHIKV) Vaccine Approaches

Vector Backbone (Strain)	CHIKV Insert	CHIKV Strain (Lineage)	Development Phase	Animal Models	Immune Response	Reference
MV (MV/Schwarz strain)	C-E3-E2-6K-E1	CHIKV 06.49 La Reunion isolate (ECSA)	Phase 1 completed	CD46^{tg}/IFNAR$^{-/-}$	nAB/IFN-γ–producing T cells in mice; protection from challenge/nAB in humans	[14, 17]
Alphavirus chimera (TC-83, EEEV, SINV)	C-E2-E1	CHIKV LR2006 OPY (ECSA)	Preclinical	NIH Swiss/ C57Bl/6; CD-1/ A129	nAB; protection from challenge	[18–20]
Adenovirus (CAdVax)	C-E3-E2-6K-E1	LR2006 OPY (ECSA)	Preclinical	CD-1/ C57Bl/6	nAB; protection from challenge	
MVA (MVA-GFP)	E3-E2-6K-E1 E3-E2 6K-E1	CHIKV-S27 (West African)	Preclinical	AG129	nAB (E3-E2-6K-E1 superior response); protection against challenge by full E cassette and E3-E2 (in the absence of nAB)	[21]
MVA (MVA-GFP)	E3-E2	. . .	Preclinical	BALB/c; A129	CD4$^+$ T-cell–mediated protection against challenge; NO nAB	[22]
MVA (MVA-GFP)	C-E3-E26K-E1	LR2006-OPY (ECSA)	Preclinical	C57BL/6	nAB; CHIKV E1 or E2–specific CD8$^+$ T cells; protection against challenge	[23]
VSVS (VSVΔG)	E3-E2-6K-E1	CHIKV S27 (West African)	Preclinical	C57BL/6	nAB; IFN-γ–producing T cells; protection against challenge	[24]
DNA (pVax1)	E1-E2-C	. . .	Preclinical	C57BL/6	Ab; IFN-γ–producing T cells	[25]
DNA (pVAX1)	E3-E2-E1	. . .	Preclinical	. . .	. . .	[26]

Abbreviations: A129 mice, type 1 interferon receptor–deficient mice; AG129, type 1 and 2 interferon receptor deficient E (3-2 or 1); C, CHIKV capsid protein; CAdVax, complex adenovirus vector; CD46tg/IFNAR$^{-/-}$, human CD46–expressing mice deficient in type 1 interferon receptor (measles virus mouse model); E, CHIKV envelope protein; ECSA, East/Central/South African lineage; EEEV, eastern equine encephalitis virus; MV, measles virus; MVA, modified vaccinia virus Ankara; nAB, neutralizing antibodies; SINV, Sindbis virus; TC-83, Trinidad donkey alphavirus strain (Venezuelan equine encephalitis virus–attenuated strain).

robust, and protective immune response in small (MV-susceptible $CD46^{tg}/IFNAR^{-/-}$ mice) and large (nonhuman primate/cynomolgus macaque) animal models.

Brandler et al described the generation and characterization of the recombinant MV/Schw-based chikungunya vaccine (MV-CHIK). The entire subgenomic open reading frame (ORF) expressing the CHIKV structural genes (C-E3-E2-6K-E1) was inserted in the intergenomic region between the MV phosphoprotein (P) and matrix protein (M). The CHIKV donor sequence was derived from CHIKV strain 06.49 (ECSA strain), a clinical isolate from the 2005/2006 La Réunion Island outbreak. The recombinant MV-CHIKV grows to wild-type titers, showing that the relatively large insert of approximately 3.7 kb did not interfere with the MV life-cycle. Upon infection of Vero cells, the structural CHIKV genes lead to the formation of virus-like particles (VLPs) in the cell-culture supernatant. The vaccine candidate induced a robust and protective humoral and cellular immune response in $CD46^{tg}/IFNAR^{-/-}$ mice after 1 or 2 immunizations. One hundred percent of the vaccinated animals were protected from lethal challenge after a single immunization, even in the presence of measles preexisting immunity. The passive transfer of MV-CHIK preimmune sera to naive mice conferred protection against a lethal challenge [17].

Based on these results and on additional nonclinical animal safety data (Themis Bioscience, unpublished data), a phase 1 clinical trial was conducted in Austria at the Medical University of Vienna. This first clinical trial was designed to test the safety, tolerability, and immunogenicity of the recombinant vaccine in the presence of preexisting anti-MV immunity (EudraCT no. 2013-001084-23). A total of 42 healthy volunteers aged 18-45 years (mean age [±SD], 30.5 ± 7.3 years), most (98%) of whom were white, were included in this randomized, observer- and subject-blinded dose-escalation trial. Thirty-six subjects received one of 3 MV-CHIK doses (1.5×10^4–3.0×10^5 50% tissue culture infection doses per dose), and 6 subjects received the control vaccine Priorix, a measles-mumps-rubella vaccine containing the parenteral MV/Schw strain. The subjects were block-randomized to receive a vaccine booster dose at 1 or 3 months after the first immunization, which allowed the assessment of short-term booster, as well as antibody persistence over 3 months.

A single immunization induced a CHIKV neutralizing antibody geometric mean titer (GMT) in all dose groups of the per protocol population (n = 36), as shown by 50% plaque reduction neutralization test ($PRNT_{50}$) [14]. The GMT after the first immunization reached 14–73, and after the second immunization the GMT reached 73 in the low-dose group and up to 433 in the high-dose group. The seroconversion rate of 40%–90% after the first immunization was boosted to 100% in all treatment cohorts after a second immunization either 1 or 3 months after the first dose. We observed no significant difference in anti-CHIKV $PRNT_{50}$ GMT between groups with low- or high-titer basal anti-MV antibodies, showing that the preexisting measles immunity did not impair a response to the transgene. The functionality of the MV vector in a preimmune population is fundamental for further development of this vector technology. Although the reason for this capacity has to be further investigated, it might be due to the replication capacity of this live vector and its capacity to be taken up by antigen-presenting cells without depending on specific receptors.

Overall, the MV-CHIK safety and tolerability profile was acceptable. The vaccine did not induce serious adverse events. The most frequently observed solicited adverse events included headache (57%), injection site pain (50%), and influenza-like illness (45%). Transient musculoskeletal pain was reported in 12% of subjects at the first visit, and, overall, 6 participants experienced local pain or erythema, headache, and pyrexia. The number of solicited local vaccine reactions increased with the dose. However, these were considered related to the high inoculation volume and the vaccine salt-buffer diluent, rather than to the active ingredient. Furthermore, no clinically relevant abnormalities in safety laboratory parameters or urinalysis results were observed [14].

The mechanism of action of the MV-CHIK vaccine is currently under investigation. However, the ability of the recombinant vaccine to potentially produce VLPs results in the formation of highly immunogenic epitopes, which is most likely the main immunological determinant for the MV-CHIK function. This is further supported by another project involving a nonreplicating CHIKV VLP vaccine. Chang et al demonstrated the vaccine potency in a phase 1 dose-escalation, open-label clinical trial in 25 adult volunteers [37]. Even in the absence of adjuvants, the vaccine induced potent neutralizing antibody responses after 1 or 2 booster doses. This vaccine and other recombinant approaches are reviewed in this issue by J. Ledgerwood et al.

The MV-CHIK vaccine is in the very early stage of development, and therefore more information will be needed before determining important details, such as target populations, including pediatric populations and subjects recently measles-vaccinated. This will need further clinical evaluation in subsequent trials. The MV-CHIK vaccine will enter a phase 2 clinical trial in 2016 to confirm the dose and schedule for further clinical development. A central aspect of this trial will be to determine the immunogenicity and reactogenicity of the vector in a larger MV preimmune population, which will be fundamental assets of the CHIKV vaccine and for the MV/Schw platform technology.

ALPHAVIRUS-BASED VACCINES

Alphaviruses are positive-stranded RNA viruses of the *Togaviridae* family. Several types of alphavirus vector systems have been engineered: replication-deficient vectors consisting of naked

RNA, recombinant single-round infectious alphavirus particles, and layered DNA vectors coding for alphavirus replicon particles [38]. All of these vector systems take advantage of the extremely efficient alphavirus RNA replication machinery that makes up to 200 000 RNA copies from each RNA molecule. For CHIKV vaccines, these vectors play a very special role, as CHIKV is also a member of the genus *Alphavirus*. Experimental alphavirus-based CHIKV vaccines are replication-competent chimeric viruses. The constructs are composed of the CHIKV structural genes combined with less pathogenic alphaviral nonstructural genes. Previous studies on neurotropic alphaviruses showed that the combination of replicative enzymes from one alphavirus with the structural proteins of another resulted in highly attenuated phenotypes in small-animal models [39]. The insertion of genetic elements that alter the virus life cycle further increased the safety of this vector system.

The nonstructural protein genes, including their subgenomic promoter and *cis*-acting promoter elements from either Venezuelan equine encephalitis virus attenuated vaccine strain TC-83, Eastern equine encephalitis, or Sindbis virus were combined with the CHIKV structural genes derived from the La Réunion strain LR2006 OPY1. All 3 chimeras induced robust neutralizing antibody titers in vaccinated C57BL/6 or National Institutes of Health Swiss mice. Even at high doses, the animals did not show any sign of neurological disease, demonstrating the preclinical safety of these vaccine candidates. The TC-83 and the naturally attenuated Eastern equine encephalitis backbones were more immunogenic than the Sindbis virus backbone [18]. To further increase the safety and potency of the vaccine candidates, their mechanisms of replication and virus host interactions were investigated. The promoters regulating the CHIKV structural genes were replaced by an encephalomyocarditis virus–derived internal ribosomal entry site (IRES). In both immunocompetent CD-1 mice and immunocompromised type 1 interferon–deficient A129 mice, a single vaccination induced robust neutralizing titers and protected these mice against CHIKV viremia and death after CHIKV challenge. An important feature regarding safety was the inhibition of virus replication in mosquito cells through the IRES, making mosquito transmission of these novel vectors impossible [40]. In another approach, an attenuated CHIKV was generated by the introduction of the IRES in the CHIKV genome, resulting in an effective and safe vaccine candidate [41]. Details are reviewed elsewhere in this issue by Weaver et al.

AD VECTOR

Ad vectors are based on a family of double-stranded DNA viruses that typically cause respiratory or gastrointestinal infections in humans. The vectors are very well described and were studied for gene therapy or vaccines in >400 clinical trials. The large safety database yielded by these trials suggests that the vaccines or gene therapy vectors are safe and well tolerated. Ad vectors can be easily produced at high titers, and vaccine formulations maintain the required stability for commercial use [42]. As for most other viral vectors, the preexisting immunity is a major concern for the development of Ad-based vaccines. The seroprevalence against Ad5 in the US population is as high as 40%–45% [11]. However, preexisting immunity might be overcome with an increased dose [13].

The construction of a recombinant Ad vector–based CHIKV vaccine and its characterization in mice was described by Wang et al [43]. The entire CHIKV ORF derived from the La Réunion isolate LR2006-OPY, encoding the structural genes, was inserted into the nonreplicating complex Ad vaccine (CAdVax) vector. CAdVax is based on the Ad5 backbone and lacks E1, E2, and most of E4. The transgene is under the control of the immediate early human cytomegalovirus promoter and the bovine growth hormone polyadenylation signal (BGHpA) sequence. Both CD-1 and C57BL/6 mice were immunized once or twice via the intraperitoneal route. High levels of CHIKV-specific antibodies were induced after a single vaccination. Neutralizing antibody titers against the homologous ECSA strain and a heterologous Asian strain reached similar high levels. In addition, vaccinated animals were challenged in the rear feet with a homologous or heterologous CHIKV strain, which induce arthritic disease as manifested by self-limiting perimetatarsal swelling [44]. A single immunization with CAdVax-CHIK protected against CHIKV viremia and joint swelling. Together, the data presented here showed a potent experimental vaccine that can be further evaluated for use in humans.

MODIFIED VACCINIA VIRUS ANKARA (MVA)–BASED VACCINES

MVA virus was derived from the Turkish smallpox vaccine strain (chorioallantois vaccinia virus Ankara) by >500 passages on chicken embryonic fibroblast cells. The newly generated strain lost nearly 30 kb of its viral genome at 6 genomic locations, which mainly impacted the hosts' response to infection [45, 46]. MVA particles initiate the viral life cycle but are unable to productively grow and generate infectious viral particles in human cells [47]. Poxvirus vectors efficiently induce heterologous antigen-specific T-cell and antibody responses in humans [48]. This family of vaccine vectors has been extensively used in humans over the past decades without raising any safety concerns.

Very recently, 3 independent studies described MVA-based CHIKV vaccine candidates in preclinical development. Martina et al describe the generation and characterization of 3 recombinant MVA viruses expressing CHIKV inserts derived from the CHIKV-S27 strain: 6KE1, E2E1, and the entire envelope protein cassette E3-E2-6K-E1. Immunization of immunocompromised AG129 mice with the 3 recombinant viruses induced neutralizing antibodies to the homologous CHIKV S27 strain. The titers

elicited by MVA-6KE1 and MVA-E3E2 were low ($PRNT_{50}$, 10–20), while the titers induced by the full envelope gene were significantly higher ($PRNT_{50}$, 40–160). Interestingly, both MVA-E3E26KE1 and MVA-E3E2 fully protected CHIKV-susceptible AG129 mice against lethal challenge with CHIKV S27 strain [21]. Similar findings were reported in another laboratory with a recombinant MVA construct expressing E3-E2 only. The selection of the E2 antigen as a single immunogen was driven by a recent study that described the E2 as the major neutralizing antigen after primary infection [49]. The coexpression of E3 peptide facilitates correct folding of the E2 protein. A total of 100% of wild-type BALB/c mice immunized with the MVA-E3-E2 vector were protected from viremia. Additionally, A129 mice lacking the type 1 IFN receptor were protected from viremia, footpad swelling, and mortality. Despite recent data suggesting that E2 would contain the main neutralizing epitope, the sera of mice immunized with MVA-E3-E2 had very low neutralizing antibody titers. In addition, the passive transfer of sera from immunized mice did not protect against lethal challenge in A129 mice. However, $CD4^+$ T cells were critical in protecting mice against challenge [22]. Together, these 2 independent studies clearly demonstrate that neutralizing antibodies can confer protection against CHIKV infections; however, a role for cellular responses cannot be ruled out. Garcia-Arriaza et al described another MVA vaccine candidate with an E3-E2-6K-E1 CHIKV insert derived from the Indian Ocean strain LR2006-OPY. This vaccine strain induced very high titers of neutralizing antibodies and a strong cellular $CD8^+$ T-cell response. The MVA-CHIK vaccine protected C57BL/6 mice against challenge in a footpad-swelling model [23].

All MVA-based CHIKV candidates described here are immunogenic and protect against CHIKV challenge in different mouse models. However, only the expression of the full structural gene cassette induced a potent neutralizing and cellular immune response. A balance of both responses is certainly the most effective approach.

VSV

VSV is a negative-stranded RNA virus of the *Rhabdoviridae* family. The virus backbone has been used as an experimental vaccine vector against several viral and bacterial pathogens. Very recently, a recombinant VSV vaccine expressing Zaire Ebola virus glycoprotein G was tested in a phase 1/2 trial involving 59 healthy volunteers [50]. The vaccine was reactogenic in humans, and the most effective dose for an acceptable immunogenicity/tolerability ratio has yet to be determined. VSV vectors lacking glycoprotein G (VSVΔG) can be used to express heterologous viral antigens to produce chimeric viruses. An experimental vaccine was designed to express the CHIKV structural genes (C-E3-E2-6K-E1) in a VSV or VSVΔG backbone. Recombinant viruses in the VSVΔG incorporated the CHIKV proteins on their surface and allowed propagation in cell culture. Mice immunized with either VSV-CHIK or VSVΔG-CHIK induced high titers of neutralizing antibodies. The VSVΔG-CHIK vaccine induced a superior antibody response. Vaccinated animals were protected against challenge in a footpad swelling mouse model, and these animals showed reduced viremia levels. In addition, the VSV-CHIK constructs induced a CHIKV E1– and CHIKV E2–specific IFN-γ–producing T-cell response after a single immunization. These data demonstrate a potent and effective vector system for the expression of CHIKV antigens. A major advantage of this system is the lack of antivector antibodies due to the lack of the G surface protein. However, the reactogenicity of this system in humans has to be further observed in detail before large-scale clinical trials can be launched.

CONCLUSION

The development of effective vaccines to prevent CHIKV infection is of great interest owing to the presence of a large CHIKV-naive population in the Americas. The strategies described here outline vaccine technologies to generate next-generation vaccines. Common to all strategies presented here is the use of replicating viruses as vaccine carriers. The experimental vaccines were potent and safe in animal models. The vaccine safety and potency has yet to be determined in the background of antivector immunity. When comparing the different CHIKV antigens tested, the data suggest that the full envelope gene cassette or the entire ORF expressing the structural genes that allow VLP formation yield the most-potent and most-protective vaccine candidates. An important aspect that has to be addressed in all of these vectored approaches is the role of preexisting antivector immunity. One of the vectored candidates, the MV/Schw vaccine, will enter phase 2 clinical trials soon, which will give valuable data on the potency and safety of this system in a larger population.

Notes

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Potential conflict of interest. K. R. is an employee of Themis Bioscience, a pharmaceutical company engaged in development and commercialization of the MV/Schw based MV-CHIK vaccine. F. T. is an employee of the Centre National de la Recherche Scientifique; he is the inventor of the MV/Schw technology and has not received personal fees from Themis Bioscience for vaccine development. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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