Next-Generation Live-Attenuated Rift Valley Fever Vaccines

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Live-attenuated Rift Valley fever (RVF) vaccines transiently replicate in the vaccinated host, thereby effectively initiating an innate and adaptive immune response. Rift Valley fever virus (RVFV)-specific neutralizing antibodies are considered the main correlate of protection. Vaccination with classical live-attenuated RVF vaccines during gestation in livestock has been associated with fetal malformations, stillbirths, and fetal demise. Facilitated by an increased understanding of the RVFV infection and replication cycle and availability of reverse genetics systems, novel rationally-designed live-attenuated candidate RVF vaccines with improved safety profiles have been developed.

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1. Rift Valley Fever

Rift Valley fever (RVF) is a mosquito-borne disease of ruminants, camelids and humans. High fatality ratios among young animals, mainly lambs and goat kids, and abortion storms in sheep flocks are characteristic features of RVF outbreaks. Whereas the RVF virus (RVFV) is transmitted between livestock primarily via mosquito vectors, humans can become infected either via mosquito bites or via contact with contaminated animal products, e.g., during the slaughtering of infected animals, contact with aborted fetal tissues and fluids, or the consumption of raw milk products. Most infected humans develop a self-limiting viral illness (characterized by fever, myalgia/arthralgia and general malaise). However, in a small percentage of cases, complications may develop, varying from retinal lesions to encephalitis and life-threatening hemorrhagic fever. The overall case fatality rate is estimated to range from 0.5% to 2%, but higher mortality ratios have been reported among hospitalized cases [1].

Following the first recorded outbreak of RVF in the 1930s, the virus caused large epizootics and epidemics in numerous countries across the African continent. Between 2020–2022, outbreaks have been reported in Burundi, Uganda, Niger, Libya, Mauritania, Senegal, Sudan, Kenya, and Madagascar. In most countries where RVFV emerged, the virus continues to cause epizootics and epidemics, generally separated by long inter-epizootic/epidemic periods. Stimulated by climate change and globalization, RVFV is expected to continue causing epidemics in previously unaffected regions [2], following in the footsteps of many other arthropod-borne viruses that have emerged in the past decades, including West Nile virus, Chikungunya virus, Zika virus, Usutu virus, Bluetongue virus, Schmallenberg virus and, most recently, Japanese encephalitis virus. Whereas most of these viruses cause disease in either animals or humans, RVFV is broadly pathogenic to a variety of species and the

cause of serious disease in three major livestock taxa (sheep, goats, cattle), camelids, wild ruminants, rodents, and humans. Furthermore, over 40 different mosquito species have been shown in laboratory studies to be able to support RVFV replication, several of which have a global distribution [3].

Outbreaks of RVFV can be massive and affect millions of animals across large geographical areas. This has resulted in the classification of RVF as a disease notifiable to the World Organization for Animal Health (WOAH, formerly known as Office International des Épizooties [OIE]). RVFV is additionally classified as an “overlap” Select Agent regulated by both the US Centers for Disease Control and Prevention (CDC) and the US Department of Agriculture (USDA) and is listed as a Category A priority pathogen by the US National Institutes of Health (NIH). RVFV is also included on the World Health Organization (WHO) R&D Blueprint list of human pathogens likely to present a large-scale risk to global health in the absence of effective countermeasures.

2. Current RVF Vaccine Landscape

Presently, only a few veterinary RVF vaccines are commercially available in a limited number of African countries. For humans, no fully-licensed commercial vaccine is available. The first live-attenuated RVFV vaccine developed for use in livestock was based on the Smithburn strain, a strain isolated by K. C. Smithburn after intracerebral passaging (>100 times) of the virulent Entebbe strain in mice [4]. The Entebbe strain originated from Uganda and was isolated from a pool of *Aedes* and *Eretmapodites* mosquitoes. Although vaccines based on the Smithburn strain are the most widely used RVF vaccines in Africa, they are considered unsafe for specific ruminant breeds and may cause teratogenic effects when administered during gestation [5][6][7].

The isolation of Clone 13, a plaque-purified clone with a naturally occurring deletion of 69% (549 nucleotides) of the NSs gene, represented another milestone in RVF vaccinology. The Clone 13 vaccine was shown to be highly efficacious in three target species (sheep, goats, cattle), including pregnant animals [8]. However, a later study in which an overdose of Clone 13 was applied in pregnant ewes showed a strong association with congenital malformations and fetal stillbirths and demonstrated that the virus could cross the placental barrier [9].

Inactivated vaccines for RVFV have also been commercialized and can be utilized safely in pregnant animals. However, the optimal efficacy of these vaccines depends on two initial immunization doses and yearly booster vaccinations. Significant progress has also been made with the development of vaccines based on viral vector platforms, although none have yet achieved licensure.

3. Current Status of Next-Generation Live-Attenuated RVF Vaccine Development

3.1. MP-12 and MP-12 Deletion Variants

Besides the Smithburn and Clone 13-based vaccines, which are commercially available for livestock, the live-attenuated MP-12 vaccine is probably one of the best-characterized RVF vaccines to date. The efficacy of MP-12-

based vaccines is outstanding with a single dose, similar to the Clone 13 and Smithburn vaccines; however, safety concerns have also been noted for this vaccine, including fetal malformation in ewes vaccinated during the first trimester [10] and mild transient hepatocellular necrosis in vaccinated lambs and calves [11][12]. Though MP-12 is not yet commercially available, the vaccine is currently conditionally licensed for ruminant livestock in the United States. MP-12 has also been evaluated in phase 1 and 2 clinical trials in human volunteers [13][14].

MP-12 was developed by the serial passage (#12) and plaque cloning of the virulent wild-type ZH548 strain in human diploid lung MRC-5 cells in the presence of the chemical mutagen 5-fluorouracil. MP-12 was found to contain four, nine, and 10 mutations in the S, M, and L segments, respectively. At least three amino acid substitutions in the M and L segments (Gn-Y259H, Gc-R1182G, and L-R1029K) were shown to contribute to the attenuation of the strain [15]. Combined reversed mutations at those three sites (Gn-H259Y, Gc-G1182R, and L-K1029R) partially recovered virulence in a mouse model, though 80% of inoculated animals survived, indicating that the attenuation phenotype of the MP-12 strain is obtained by a combination of multiple mutations, including those three sites. Notably, MP-12 replication is restricted at 38 °C and above due to four temperature-sensitive mutations in the M and L segments (Gn-Y259H, Gc-R1182G, L-V172A, and L-M1244I) [16]. Among them, the L-M1244I mutation was not stable following 25 serial viral passages at 37 °C in Vero or MRC-5 cells [17]. Consequently, the genetic integrity of the MP-12 vaccine should be ensured by maintaining an appropriate seed lot system and culture temperature.

For all RVFV live-attenuated vaccine candidates, including MP-12, mosquito-borne transmission has been cited as a potential concern [18]. However, although the MP-12 strain retains the ability to infect and disseminate in mosquitoes via the oral intake of blood meals, transient low-level viremia (~10^3 PFU/mL) in vaccinated animals is not considered sufficient for viral transmission to and dissemination in mosquitoes [18][19]. Furthermore, if reassortant strains emerge, which is so far only a theoretic possibility, through the combination of MP-12 and a circulating wild-type RVFV in animals or mosquitoes, the reassortant would have a virological phenotype like that of the circulating wild-type RVFV and would not create an additional safety risk [20]. A comprehensive overview of the safety and efficacy of MP-12-based vaccine candidates can be found in the following review paper [21].

To improve the safety profile of MP-12, several research groups constructed MP-12 variants lacking one or more of the virulence genes using reverse genetics or even reconfigured the genome to a two-segmented variant [22][23][24]. Two MP-12 variants referred to as arMP-12ΔNSs16/198 and arMP-12ΔNSm21/384, which lack an intact NSs and P78/NSm gene, respectively, could induce protective immunity in 3 to 4-month-old young sheep, 4 to 6-month-old cattle, or pregnant ewes without safety concerns [25][26][27]. Notably, the neutralizing antibody responses following inoculation with arMP-12ΔNSm21/384 were stronger compared to those induced by arMP-12ΔNSs16/198 [28]. A large-scale follow-up experiment with 17 pregnant African domestic sheep vaccinated with arMP-12ΔNSm21/384 during the early stage of pregnancy (<35 days gestation) resulted in fetal malformations (forelimb malformation and deformed tail) in 18% of vaccinated ewes [28]. At post-mortem, the tissues of dead lambs (spleen, lung, brain, and long bone) were negative for RVFV as analyzed by PCR. Nevertheless, these results indicated that the arMP-12ΔNSm21/384 vaccine should not be used in pregnant sheep during the first month of gestation.

More recently, the next-generation MP-12 strain, RVax-1, was generated and characterized [29]. The RVax-1 candidate vaccine encodes 36, 37, 167, and 326 silent mutations in the N, NSs, M, and L ORFs, respectively, in the backbone of arMP-12ΔNSm21/384. A cluster of silent mutations was introduced at every 50 nucleotides within the ORFs, while the codon usage or codon pair bias was not deoptimized to maintain efficient viral translation. All 566 silent mutations were genetically stable across 10 serial passages in Vero cells, and especially the silent mutations in the S- and L-segment were shown to strengthen the attenuated phenotype, compared to MP-12 [30]. Following direct recovery from Vero cells using polymerase I-based reverse genetics [31], RVax-1 was shown to replicate efficiently in Vero and MRC-5 cells and to have comparable immunogenicity and protective efficacy compared to the full-length rMP-12 strain in a mouse model. Moreover, the RVax-1 strain, but not the full-length rMP-12, was shown to poorly disseminate in orally fed *Aedes aegypti* mosquitoes, indicating a minimum risk of mosquito-borne transmission of RVax-1 [29]. The silent mutations unique to RVax-1 could furthermore serve as a genetic marker to trace vaccine RNA in mosquitoes and livestock animals. Further evaluations of vaccine safety, immunogenicity and efficacy will support the development of this next-generation MP-12 candidate vaccine.

3.2. DDvax

Stimulated by the 2006–2007 East Africa RVF epidemic [32], an effort to improve upon the safety and efficacy of conventional RVFV vaccines using reverse genetics systems was undertaken with the goal of developing the first rationally designed RVF vaccine candidate. Using a precise whole gene deletion strategy, the two main RVFV virulence factor genes (the NSs and NSm coding regions) were removed from the parental ZH-501 strain. These deletions resulted in a highly attenuated and highly immunogenic vaccine virus particle initially designated as DNSs-DNSm-rZH501, and is currently referred to simply as “DDvax” for “double-deletion vaccine” [33].

Initial testing in rats revealed a high safety profile with no obvious clinical adverse events after inoculation of DDvax at doses exceeding 100,000× the known lethal dose 50 (LD_{50}) of the parental RVFV strain. Complete protection from high-dose virulent virus challenge was observed 28 days post-vaccination. Over the past 16 years since that initial study, DDvax has been proven safe, immunogenic, and effective in preventing virulent virus infection and disease following a single dose administration in a variety of animal species, including multiple rodent species, adult pregnant and non-pregnant livestock species, and two species of nonhuman primates (marmosets and rhesus macaques) [34][35][36] (and manuscripts in preparation). In each animal species tested, robust and rapid rises in neutralizing antibodies were observed from 14 to 21 days post-vaccination. Surprisingly, results in rodents have indicated that rapid protection from virulent virus infection is conferred in as little as 2–3 days post-vaccination (manuscript in preparation). This early protection was observed before the onset of detectable neutralizing antibodies and may be conferred by the stimulation of robust innate cellular responses (e.g., interferon and other mediators) linked to de novo vaccine replication and protein synthesis.

Further preclinical and research and development activities with the underlying DDvax technological platform have also revealed insights into RVFV mosquito infection and transmission factors using various single (NSs or NSm only) and double (NSs and NSm) whole gene deletion recombinant viruses. As part of the safety assessments of DDvax and these other gene deletion RVF viruses, initial mosquito transmission experiments revealed that the

NSm gene (and/or P78) confers critical mosquito mid-gut barrier evasion mechanisms that, when deleted, provide an additional environmental containment safety factor. Across several studies, the DDvax research team has definitively demonstrated that DDvax cannot be efficiently transmitted by multiple mosquito vectors due to the specific deletion of the NSm gene region [37][38][39]. These results, when taken together, indicated that DDvax might provide in a single dose rapid, safe, and robust protection from RVFV infection with an excellent environmental safety profile that would potentially enable use in both routine and emergency outbreak response-related vaccination campaigns.

3.3. RVFV-4s

RVFV-4s comprises four instead of the natural three-genome segments, resulting from splitting the M-segment into an M-type segment encoding NSm and Gn and an M-type segment encoding Gc. Several RVFV-4s variants have been constructed, including variants encoding eGFP [40], though for vaccine purposes, two RVFV-4s strains have been brought beyond the proof-of-concept phase: vRVFV-4s for veterinary use and hRVFV-4s for human use. vRVFV-4s is based on wild-type strain 35/74 isolated from a sheep, whereas hRVFV-4s is based on Clone 13, which was derived from wild-type strain 74HB59, isolated from a human case. Both vaccine candidates lack an intact NSs gene, in addition to a split M-segment. The vaccine viruses were shown to be genetically stable (>20 passages), to not revert to virulence and to not disseminate within a mammalian host or spread to the environment [41].

The attenuated phenotype of RVFV-4s, comprising four genome segments instead of three, is explained by the lower chance of incorporating a complete set of genome segments and an imbalance in replication and transcription [40]. A single vaccination with vRVFV-4s was shown to induce robust levels of neutralizing antibodies within 1–2 weeks after vaccination in all species evaluated (sheep, goats, cattle) [41]. The initial safety of the hRVFV-4s vaccine was assessed in mice following intranasal administration. In contrast to Clone 13, which can induce lethal encephalitis when administered intranasally in mice, hRVFV-4s was shown to be completely avirulent and unable to cause viremia [42]. High-dose experiments with young lambs (intramuscular route), which are the most susceptible target animals of RVFV, subsequently demonstrated an absence of viremia, absence of shedding and spreading and confirmed that RVFV-4s could not be passaged from animal to animal and does not revert to virulence [41]. Furthermore, a recent experiment with marmosets, a nonhuman primate species highly susceptible to the wild-type virus, showed that vRVFV-4s and hRVFV-4s do not induce untoward effects nor disseminate beyond draining lymph nodes [43]. Finally, RVFV-4s was shown to be safe for pregnant ewes, with the absence of vertical transmission during the first trimester of gestation [44]. Additional studies assessing (reproductive) toxicology and potential virus dissemination in mosquitoes are pending.

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