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Towards a safe, effective vaccine for Rift Valley fever virus

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Abstract

Rift Valley fever virus (RVFV) is an important animal and human threat and leads to longstanding morbidity and mortality in susceptible hosts. Since no therapies currently exist to treat Rift Valley fever, it remains a public and animal health priority to develop safe, effective RVFV vaccines (whether for animals, humans, or both) that provide long-term protective immunity. In the evaluated article, Bhardwaj and colleagues describe the creation and testing of two successful vaccine strategies against RVFV, a DNA plasmid vaccine expressing Gn coupled to C3d, and an alpha-virus replicon vaccine expressing Gn protein. Both vaccines elicited strong neutralizing antibody responses, prevented morbidity and mortality in RVFV-challenged mice, and enabled protection of naive mice via passive antibody transfer from vaccinated mice. Both DNA and replicon RVFV vaccines have previously been shown to protect against RVFV challenge, but these results allow for direct comparison of the two methods and evaluation of a combined prime–boost method. The results also highlight the specific humoral and cell-mediated immune responses to vaccination.

Keywords

Rift Valley Fever; vaccine

Summary of methods

In their study, Bhardwaj and colleagues performed experiments to directly compare DNA vaccines and alphavirus vaccines expressing Rift Valley Fever virus (RVFV) Gn, evaluate the efficacy of these DNA vaccines against Rift Valley Fever (RVF) in a mouse model, determine whether a prime–boost strategy enhances efficacy, and assess the nature of the immune response to vaccination [1]. RVFV is a member of the *Phlebovirus* genus, one of the five genera in the family *Bunyaviridae* [101]. RVFV, similar to other Bunyaviruses, has a single-stranded, tripartite-negative or ambisense-coded RNA genome, composed of the L, M and S segments encoding four structural proteins, viral polymerase (L segment), glycoproteins (M segment) and nucleocapsid protein N (S segment) [2]. RVFV is a high-priority pathogen because of its ability to cause blindness, encephalitis and life-threatening hemorrhagic fever in humans [3,102], its potential for severe economic harm to livestock [101], and its potential for nonvector aerosol spread during epizootics and epidemics [4,5].

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Vaccination of both humans and animals against RVFV could enable prevention of an emerging worldwide threat.

In order to construct the DNA plasmid vaccine tested in this study, the Gn glycoprotein from RVFV isolate ZH548 was cloned into the eukaryotic expression vaccine vector pTR600, and amplified using an *Escherichia coli* DH5α system. Alternatively, Gn was fused to three tandem repeats of the mouse homolog of C3d. To prepare the replicon vaccine, a soluble form of RVFV Gn was introduced behind the 26S subgenomic promoter of the Venezuelan equine encephalitis virus replicon plasmid pVR21, and transcripts were electroporated into BHK-21 cells to package replicon particles. Female BALB/c mice aged 6–8 weeks old were used for the vaccine studies. Gene gun experiments were performed on shaved abdominal skin using the Bio-Rad delivery system. DNA vaccines were administered three times at 3-week intervals. Replicon mice were given one dose at week 6, or three doses at 3-week intervals (weeks 0, 3 and 6). Blood samples were collected at baseline and 2 weeks after each vaccine dose. For comparison, mice were immunized with RVFV MP12, a live-attenuated vaccine, at 2 or 8 weeks before challenge. MP12 and ZH501 (the attenuated and virulent strains used for challenge experiments, respectively) were propagated and titrated in Vero cells. A whole-virus inactivated preparation (WIV MP12) was also made, and administered to mice three times at 3-week intervals.

To evaluate the humoral response to vaccination, indirect ELISAs using inactivated RVFV MP12 as the coating antigen, were performed on sera to assess the anti-Gn IgG response. Neutralizing antibodies to RVFV ZH501 were measured using plaque-reduction neutralization testing. ELISpot assays were performed to identify the number of anti-Gn-specific murine INF-γ-secreting splenocytes.

To assess protection from infection, challenge experiments using 1×10^3 RVFV ZH501 were undertaken in BSL 3-enhanced laboratories. Animals were examined twice daily for visual signs of morbidity of mortality, using a laboratory-validated scoring system. Finally, sera from vaccinated mice were transferred to naive BALB/c mice, and 1 h later, the mice underwent virulent RVFV ZH501 challenge. These mice were observed daily for 8 days post-transfer for signs of morbidity or mortality.

Summary of results

Both DNA plasmid vaccines (Gn and Gn-C3d) and the Gn replicon vector vaccine (Rep-Gn) were efficiently secreted from transfected cells, and produced anti-Gn antibody responses in mice after three vaccinations. Rep-Gn had the greatest titer (1:2560) followed by Gn-C3d (1:1280) and Gn (1:180). Mice vaccinated with Gn DNA only, or who received only one DNA vaccine, did not elicit any detectable anti-Gn antibodies. DNA prime–replicon-boosted mice were vaccinated twice with Gn-C3d-DNA, and then administered a single inoculation of replicon expressing Gn, and had higher anti-Gn antibody titers (1:4160) compared with mice vaccinated with a single vaccination of alpha virus replicon (1:280). MP12 vaccination elicited a mixed Th1 and Th2 response, whereas mice vaccinated with three doses of WIV MP12 had a Th2-restricted immune response. Mice vaccinated with Gn-C3d-DNA vaccines elicited predominately IgG1, whereas replicon vaccination elicited not only IgG1, but also IgG2a and IgG2b isotypes, similar to those elicited by the live-attenuated MP12 vaccine. Mice primed with Gn-C3d-DNA maintained an IgG1 isotype bias following replicon boost.

At 2 weeks following the third vaccination, mice receiving Gn-C3d- or Rep-Gn-neutralized RVFV ZH501. Those who received the DNA prime–replicon-boost vaccine did not have significantly enhanced neutralizing titers. Mice vaccinated with the MP12 vaccine strain had the highest neutralizing titers (average 1:656–1:736), and they were significantly higher than

sera from mice vaccinated with Gn, Rep-Gn and WIV MP12. Those vaccinated with WIV MP12 showed low neutralizing titers.

Vaccinated mice were challenged with MP12 virus 2 weeks after the last immunization, and 6 days later splenocytes were collected and stimulated *in vitro* with eight overlapping pools of peptide (15-mers overlapping by 11) specific for Gn. Mice vaccinated with Rep-Gn or Gn-C3d/Rep-Gn had responses to a stretch of 111 amino acids, starting at amino acid 53 in the Gn sequence. Mice vaccinated with DNA vaccines did not elicit cellular responses. Four potential peptides (18, 19, 36 and 38) were identified as responsible for the vaccine-elicited cellular responses, and were predicted to be MHC class I restricted.

All the mice vaccinated with MP12, Gn-C3d-DNA, Rep-Gn, or in a DNA prime–replicon-boost strategy were protected from mortality and morbidity after challenge, 2 weeks after final vaccination, with a lethal dose of RVFV ZH501. In total, 60% of mice that received Gn without the molecular adjuvant C3d displayed ruffled fur and lethargy, and one mouse died. Mice vaccinated with WIV MP12 were not protected from challenge. A few DNA control mice also survived challenge. Pooled antiserum from each vaccinated group was transferred (intraperitoneally) into unimmunized mice, which were then challenged with a lethal dose of RVFV ZH501 (Table 4 of evaluated paper). Of the mice that received sera from MP12 or Gn-C3d, 80% survived challenge, whereas 40% were protected from DNA prime–replicon-boosted or Rep-Gn sera.

Future perspective

The development of RVFV vaccines that will yield highly effective, long-term protective immunity is paramount. The ideal vaccine would be safe to administer without any pathogenic potential, confer protection against morbidity and mortality after a single dose, have the ability to ‘differentiate between naturally infected and vaccinated animals’ (DIVA), and be easy to produce in standard vaccine facilities, with a long shelf-life at ambient temperatures. The vaccine strategies in the evaluated paper achieve neutralizing immunity and protection against morbidity and mortality after challenge, and would allow for DIVA, but require multiple doses. Previous studies have shown that DNA [6] and replicon [7] RVFV vaccines protect against virulent RVFV challenge, but the Bhardwaj *et al.* compares them directly, and evaluates a combined prime–boost method. The tested vaccine strategies also protected against a virulent strain of RVFV different, yet still closely related to, the strain used to construct the DNA plasmids, and may offer broad protection against different RVFV strains. The new vaccines were not superior to the live-attenuated vaccine strain MP-12; however, response to the DNA plasmid–replicon-boost strategy did mimic the Th1 and Th2 responses seen with MP12. The novel finding that the C3d molecule acted as an adjuvant and improved vaccine efficacy may have widespread applications.

Current RVFV vaccine strategies include development of safer, live-attenuated and genetically engineered RVFV strains [8], testing of chimeric alphavirus–RVFV replicon constructs [9], viral DNA-based vaccines [10], and approaches based on the production of recombinant viral proteins within virus-like particles [11]. Live-attenuated vaccines are highly immunogenic, and do not require boosting, but may have safety concerns [12,13]. Live-attenuated strains of RVFV have been used for vaccination of susceptible livestock, but can lead to miscarriage and teratogenicity among pregnant animals [9,12]. A new reverse-engineered live-attenuated vaccine candidate, rZH501ΔDNSs:GFPΔDNSm, created by replacing viral virulence genes, *NSs* and *NSm*, with green fluorescent protein, is highly attenuated, and results in high neutralizing antibody titers [8]. Killed inactivated RVFV vaccines are safe and protective but require multiple booster immunizations to achieve and maintain protection [10–12]. Replicon and viral-like particle approaches cannot revert to

virulent forms and allow for DIVA, but are expensive to produce. In addition, in replicon vaccination, antivector immunity may develop and usurp efficacy if booster vaccination is necessary. In this study, Venezuelan equine encephalitis virus was used as the vector, and is a better choice than adenovirus, since the general US population would have little pre-existing immunity. Also, the DNA prime–replicon-boost strategy tested would provide a heterologous system that may be able to overcome antivector immunity for booster vaccinations.

Executive summary

Objectives

□ The objectives were: to develop and test the efficacy of DNA plasmid and alphavirus replicon vaccines expressing the Gn glycoprotein, to test whether Gn fusion to three copies of murine C3d enhanced vaccine response, to evaluate the nature of the humoral and cell-mediated responses to vaccine and to evaluate vaccine efficacy using measures of morbidity and mortality.

Methods

□ DNA plasmid and replicon vaccines were generated.

□ BALB/c mice were immunized three times at 3-week intervals.

□ Isotype-specific IgG response, neutralizing antibody response and anti-Gn cell-mediated immune response at 8 weeks following primary vaccination were evaluated.

□ Vaccine efficacy via Rift Valley fever virus (RVFV) ZH501 challenge 2 weeks after final vaccination were demonstrated, and post-challenge morbidity and mortality were monitored.

□ Passive sera transfer to naive RVFV-challenged mice were evaluated in each vaccine group.

Results

□ C3d fusion enhanced antibody response to Gn.

□ The predominant antibody isotype elicited by DNA and replicon immunizations was IgG1; however, replicon vaccine also elicited isotypes IgG2A and IgG2B.

□ Both Gn-C3d (DNA) and Gn-C3d/Rep-Gn (DNA prime–replicon boost) vaccines elicited strong neutralizing antibody responses, and protected mice against virulent RVFV challenge.

□ Passive sera transfer from vaccinated mice protected naive mice from RVFV challenge.

Conclusion

□ Both DNA plasmid and DNA prime–replicon-boost vaccines confer protection, and prevent clinical signs of infection with RVFV.

□ The addition of C3d enhanced protective efficacy of the DNA plasmid-based vaccine and merits further study.

The authors have also created Sindis virus replicon vectors expressing RVFV Gn, Gc, and NSm proteins which induce protective antibody responses in immunized mice and sheep and provide 100% protection against lethal RVFV challenge [9]. RVFV-like viral-like particles

have been produced efficiently by reverse genetics [14], and have been shown to be highly immunogenic and protective against lethal challenge in mice [11]. Other DNA gene gun immunizations with cDNA encoding RVFV structural proteins (G1 and G2) have been shown to induce neutralizing antibody titers; however, unlike the mice in this study, some immunized mice still developed clinical signs of infection after sublethal challenge [10].

In summary, multiple effective vaccine strategies will likely be necessary to combat a complex pathogen, such as RVFV. Bhardwaj and colleagues have taken a crucial step in comparing and contrasting different vaccine strategies against RVFV that were protective in a mouse model. Further studies are needed to evaluate the durability and duration of this protection in other animal models.

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