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The use of mice lacking type I or both type I and type II interferon responses in research on hemorrhagic fever viruses. Part 2: Vaccine efficacy studies

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Abstract

For more than 20 years, researchers have used laboratory mice lacking type I or both type I and II interferon (IFN) responses to study high-containment viruses that cause hemorrhagic fevers (HF) in humans. With the exception of Rift Valley fever virus, agents that cause viral HF in humans, such as Ebola and Lassa virus, do not cause disease in mature immunocompetent mice. In contrast, IFN-deficient mice typically develop severe or fatal disease when inoculated with these agents. The sensitivity of IFN-deficient mice to disease has led to their widespread use in biocontainment laboratories to assess the efficacy of novel vaccines against HF viruses, often without considering whether adaptive immune responses in IFN-deficient mice accurately mirror those in immunocompetent humans. Failure to recognize these questions may lead to inappropriate expectations of the predictive value of mouse experiments. In two invited articles, we investigate these questions. The present article reviews the use of IFN-deficient mice for assessing novel vaccines against HF viruses, including Ebola, Lassa, Crimean-Congo hemorrhagic fever and Rift Valley fever viruses. A companion paper examines the general question of how the lack of IFN signaling may affect adaptive immune responses and the outcome of vaccine studies in mice.

Keywords

IFN-Deficient mice; Ebola virus; Lassa virus; Crimean-Congo hemorrhagic fever virus; Rift valley fever virus

1. Introduction

Immunocompetent laboratory mice do not become ill when infected with Ebola virus (EBOV), Marburg virus (MARV), Lassa virus (LASV), and other viruses that cause hemorrhagic fevers (HF) in humans, but genetically modified mice lacking functional type I interferon (IFN) or both type I and type II IFN responses typically develop severe or fatal disease when inoculated with these agents. The sensitivity to disease of IFN-deficient mice,

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allowing testing of HF viruses without serial adaptation in rodents, has led to their widespread use in efficacy studies of novel vaccines against HF viruses. However, these studies often lack consideration for whether adaptive immune responses in IFN-deficient mice accurately mirror those in immunocompetent humans, which may lead to inappropriate expectations of the predictive value of mouse experiments.

In two articles, we examine the use of mice lacking type I or both type I and type II IFN responses for research on vaccines against HF viruses. The first paper examines interactions between type I and type II IFN responses, development of adaptive immunity, and the potential influence of IFN deficiency on the protective efficacy of vaccines (Clarke and Bradfute, 2020, in press). The present article reviews the published literature regarding assessment of candidate vaccines against highly pathogenic HF viruses. We provide a comprehensive summary of published reports evaluating experimental vaccines against filoviruses (EBOV and MARV), arenaviruses (LASV and South American agents), and bunyaviruses (Crimean-Congo hemorrhagic fever virus [CCHFV] and Rift Valley fever virus [RVFV]) in mice lacking the signal transducer and activator of transcription ($STAT1^{-/-}$) protein, the cell-surface type I α/β IFN receptor ($IFNAR^{-/-}$; also termed in the literature as A129), or both the type I and type II receptors (IFN $\alpha/\beta/\gamma R^{-/-}$; also termed in the literature as AG129).

2. Background

2.1. Interferons and IFN-deficient mice

Interferons, a multigene family of inducible cytokines, are categorized into 3 types: type I (IFN-α and IFN-β), type II (IFN-γ), and type III (IFN-λ1, IFN-λ2, and IFN-λ3). Targeted gene deletions in mouse embryonic stem cells have been used to produce mutant mice, including those with specific deficiencies in the IFN response cascade (Meraz et al., 1996). Viral HF vaccine studies have utilized mice with deletions in type I, II, and III IFN signaling ($STAT1^{-/-}$) or with impaired initiation of the IFN response due to receptor deletions, either in the IFN-α/β receptor alone ($IFNAR^{-/-}$) or in both IFN-α/β and IFN-γ receptors (IFN $\alpha/\beta/\gamma R^{-/-}$). In addition, induction of transient immunosuppression in mice with an IFN receptor-binding monoclonal antibody has been demonstrated as an alternative approach to using IFN-deficient mice (Garrison et al., 2017; Pinto et al., 2011; Smith et al., 2017; Teijaro et al., 2013).

2.2. Outcomes of infection of IFN-deficient mice

HF virus infection in IFN-deficient mice can cause a spectrum of mild to severe disease. However, despite immune deficiencies, asymptomatic infection has also been described, and disease can be specific to delivery route and dose of the virus (Bray, 2001). Mild disease is often limited to transient weight loss; other signs include huddling, hunched posture, and ruffled fur. Severe disease signs may include lack of responsiveness to stimuli, significant hypoactivity and/or weight loss (> 20%), or neurologic abnormalities (ataxia, circling, head pressing, paresis, or paralysis). Although variations occur depending on virus species, strain, and inoculation route and dose, end-stage disease in IFN-deficient mice usually occurs within 2 weeks, typically within the first 5–7 days after infection.

2.3. Types of vaccine platforms

Viral HF vaccine platforms tested to date include attenuated virus, recombinant and attenuated heterologous or homologous virus vectors, inactivated virus, viral proteins or protein subunits, plasmid DNA, non-replicating viral vectors, viral replicon particles (VRPs), and virus-like particles (VLPs). Each platform offers potential advantages and disadvantages in production efficiency and cost, storage and shelf life, acceptance of platform, vaccine safety, dosing, and protective efficacy (Table 1). In general, advantages of attenuated viral vaccines include induction of effective humoral and cellular immune responses against native viral proteins after a single dose, ease of production, and longevity of protection. Disadvantages are largely centered on product instability and safety concerns. Advantages of inactivated viruses, which are widely used, are efficacy and safety. However, disadvantages include the need for production in high containment (culturing, inactivation, and safety testing), denaturation of antigens during inactivation, and the need to administer multiple doses for protection.

Viral protein and subunit vaccines offer an excellent safety profile, do not require inactivation or high-containment laboratories, are accepted vaccine platforms, and are often efficient to produce. However, the protective efficacy they elicit can be lower than that of inactivated or attenuated viruses due to the lack of complex antigens and altered protein post-translational modifications. Multiple vaccine doses are often required to achieve protection. Plasmid DNA also offers an excellent safety profile and production efficiency, and post-translational modifications of plasmid DNA-derived proteins are similar to those seen in native viral proteins, but multiple doses are generally required to achieve effective immune responses. Moreover, no DNA vaccines have yet been approved for human use.

Viral vector vaccines can be either replicating or non-replicating. Non-replicating viral vectors, VRPs, and VLPs can elicit effective immune responses, often display native viral proteins, have been licensed for use in humans, and do not require inactivation or high-containment labs for production, but may require multiple doses and can be laborious to produce in high quantities.

3. Filoviruses

3.1. Susceptibility of IFN-deficient mice to filoviruses

Immune-competent mice are resistant to disease from infection with wild-type filoviruses, and therefore preliminary screening of vaccine candidates is often performed in mice using mouse-adapted (MA) virus (Bray et al., 1998) or IFN-deficient mouse strains (Bray, 2001). MA-EBOV is uniformly lethal in IFN-deficient mice, whereas infection with wild-type filoviruses does not always result in uniform lethality, depending on the viral species, strain, and mouse background (Table 2). Susceptibility to disease by wild-type filovirus infection has been reported in STAT1$^{-/-}$, IFNAR$^{-/-}$, IFNγR$^{-/-}$, and IFN $\alpha/\beta/\gamma$R$^{-/-}$ mice.

3.2. Filovirus vaccines tested in IFN-deficient mice

Filovirus vaccines against EBOV and Sudan virus (SUDV), including attenuated virus vaccines, VRPs, VLPs, and non-replicating virus vaccines, have been evaluated in STAT1$^{-/-}$

and IFNAR$^{-/-}$ mice with both homologous and heterologous challenge (Table 3), as detailed below.

3.2.1. Attenuated virus vaccines—Attenuated viruses have been evaluated in STAT1$^{-/-}$ mice. EBOVΔVP30 is a replication-deficient/incompetent virus based on the Mayinga strain of EBOV that lacks the coding region for the essential viral transcription activator, VP30. It replicates to high titers in cell lines that stably express the VP30 protein and is genetically stable (Halfmann et al., 2008). EBOVΔVP30 was safety tested in STAT1$^{-/-}$ mice (Halfmann et al., 2009, 2008) and caused no disease signs. Efficacy was not evaluated in IFN-deficient mice; studies successfully showing efficacy were performed in immunocompetent mice and guinea pigs with rodent-adapted viruses (Halfmann et al., 2009). Mice and guinea pigs immunized twice with EBOVΔVP30 were fully protected against a lethal challenge with mouse- or guinea pig–adapted EBOV, respectively. Later, the vaccine was shown to confer complete protection against wild-type EBOV Kitwit in cynomolgus macaques (Marzi et al., 2015).

3.2.2. VRP vaccines—Venezuelan equine encephalitis (VEE) VRP-based vaccines for both EBOV and SUDV have been evaluated in IFNAR$^{-/-}$ mice. Following a single vaccination with 10^6 or 10^7 infectious units (IFU) of VEE-EBOV GP VRP, IFNAR$^{-/-}$ mice seroconverted against EBOV and were fully protected from disease and lethal outcome (as measured by weight loss and survival, respectively) upon EBOV challenge (Brannan et al., 2015). Vaccination with a single low dose of VEE-SUDV GP VRP (10^3 IFU) partially protected IFNAR$^{-/-}$ mice from SUDV challenge (90%), and a 10^4–10^7 IFU dose fully protected these mice, although weight loss was still observed upon challenge.

3.2.3. VLP vaccines—VLPs consisting of the EBOV nucleoprotein (NP), glycoprotein (GP), and VP40 have been evaluated as protective vaccines in STAT1$^{-/-}$ mice. Following a prime/boost/boost inoculation regimen, STAT1$^{-/-}$ mice seroconverted against EBOV, but were not protected from MA-EBOV challenge despite delayed mean time to death (Raymond et al., 2011).

3.2.4. VSV vaccines—Replication-competent recombinant vesicular stomatitis virus (VSV) vaccines encoding EBOV or MARV GP in place of the VSV glycoprotein have been evaluated in STAT1$^{-/-}$ mice. However, unlike the EBOVΔVP30 platform, VSV expressing EBOV, MARV, or Reston virus GP causes acute lethal disease in the first week after inoculation in these animals (Marzi et al., 2015b). Thus, despite the success of replicating VSV as vaccines against EBOV in multiple animal models and humans, STAT1$^{-/-}$ mice were determined to be inappropriate for testing replication-competent VSV vaccines. While replication-competent VSV vaccines cause a lethal disease in STAT1$^{-/-}$ mice, VSV lacking the native glycoprotein (ΔG) and pseudotyped with EBOV or SUDV GP offer a non-replicating alternative and are safe (non-lethal) and immunogenic in IFNAR$^{-/-}$ mice (Lennemann et al., 2017). Three versions of VSV pseudotyped with EBOV GP have been evaluated:

1. Wild-type GP (VSV-GP)

2. GP with the first 7 N-terminal N-linked glycosylation sites abolished (VSV-GP 7G)
3. GP with all 15 N-linked glycosylation sites abolished (VSV-7Gm8G)

After a vaccine dose titration study in C57BL/6 mice receiving a prime/boost of each version demonstrated protection against mouse-adapted virus when administered at high doses, studies were conducted in IFNAR$^{-/-}$ mice challenged with wild-type EBOV and SUDV. IFNAR$^{-/-}$ mice were given 2×10^7 single-round infectious particles (SRIPs) of VSV-GP, VSV-GP 7G, or VSV-GP 7Gm8G in a prime/boost regimen (Lennemann et al., 2017). Weight loss was selected as a measure of efficacy in these studies based on previous work demonstrating significant weight loss in IFNAR$^{-/-}$ mice upon wild-type SUDV or EBOV infection. Protection against weight loss was demonstrated in IFNAR$^{-/-}$ mice challenged with EBOV. However, consistent with studies evaluating heterologous protection against SUDV after vaccinating non-human primates with a VEE-EBOV VRP vaccine (Herbert et al., 2013), vaccinated IFNAR$^{-/-}$ mice were not protected from weight loss upon challenge with SUDV (Lennemann et al., 2017).

4. Arenaviruses

4.1. Susceptibility of IFN-deficient mice to arenaviruses

With the exception of CBA mice inoculated intracranially, as shown for LASV (Uckun et al., 2004), immunocompetent mice are generally resistant to disease from experimental arenavirus infection. Only IFN-deficient mice are susceptible to disease from New World (Machupo virus [MACV], Junin virus [JUNV], and Tacaribe virus) and Old World (LASV) arenavirus infection. JUNV and Tacaribe virus cause lethal disease in IFN-α/β/γR$^{-/-}$ mice (Kolokoltsova et al., 2010; Gowen et al., 2010), whereas MACV is lethal in both IFNAR$^{-/-}$ and IFN-α/β/γR$^{-/-}$ mice (Bradfute et al., 2011; Koma et al., 2016, 2015). STAT1$^{-/-}$ mice succumb to LASV infection (Yun et al., 2013; Yun et al., 2015), but IFNAR$^{-/-}$ and IFN-α/β/γR$^{-/-}$ mice develop non-lethal disease, with more severe clinical signs reported in the former (Yun et al., 2012). More recently, capitalizing on the IFNAR$^{-/-}$ background, a unique chimeric mouse model demonstrating susceptibility to LASV has been developed by transplanting wild-type bone marrow progenitor cells into irradiated IFNAR$^{-/-}$ mice to aid studies of LASV immunity (Oestereich et al., 2016).

4.2. Arenavirus vaccines tested in IFN-deficient mice

To date, arenavirus vaccine studies in IFN-deficient mice have been limited to IFN-α/β/γR$^{-/-}$ mice infected with MACV (Koma et al., 2015). Despite the susceptibility of STAT1$^{-/-}$ mice to infection, no LASV vaccine studies have been reported in these or any other mice with impaired IFN signaling (Table 4).

4.2.1. Live attenuated vaccine—rMACV/Cd#1-GPC, a recombinant MACV containing the glycoprotein of the attenuated JUNV strain Candid#1, is avirulent in IFN-α/β/γR$^{-/-}$ mice. Following intraperitoneal (IP) inoculation with 10^5 plaque-forming units (PFU) of this recombinant virus, no weight loss, increased body temperatures, or disease signs were observed in the mice. In addition, no evidence of virus was found by plaque

assays 17 or 42 days post infection (dpi) in brain, spleen, liver, or serum samples. rMACV/Cd#1-GPC was evaluated as a vaccine candidate against wild-type MACV in IFN-α/β/γR$^{-/-}$ mice given an immunization dose of 10^4 PFU IP. IFN-α/β/γR$^{-/-}$ mice vaccinated with rMACV/Cd#1-GPC seroconverted against MACV antigen, and were protected from weight loss and lethal disease following IP challenge with 10^4 PFU of MACV. Furthermore, no infectious virus or pathological changes were detected in brains, livers, or spleens of vaccinated IFN-α/β/γR$^{-/-}$ mice. In contrast, although a statistically significant delay in time to death was observed, none of the IFN-α/β/γR$^{-/-}$ mice vaccinated with wild-type attenuated JUNV Candid#1 survived MACV challenge. Similarly to mock-vaccinated mice, virus was detected in brain, spleen, liver, and serum samples of JUNV Candid#1-vaccinated mice along with comparable pathologic changes (endothelial hypertrophy and vascular mononuclear infiltrates in the brain, and microvesicular steatosis and perivascular mononuclear infiltrates in the liver) (Koma et al., 2015).

5. Nairoviruses

5.1. Susceptibility of IFN-deficient mice to nairoviruses

Both STAT1$^{-/-}$ and IFNAR$^{-/-}$ mice are highly susceptible to disease from CCHFV infection (Bente et al., 2010; Bereczky et al., 2010; Zivcec et al., 2013) (Table 5), with a 50% lethal dose (LD50) of 4 PFU upon IP delivery and 0.05 of 50% tissue culture infective dose ($TCID_{50}$) upon subcutaneous (SC) infection with the IbAr10200 strain. Most studies have used the IbAr10200 strain (Table 6), isolated from *Hya-lomma excavatum* ticks collected from a camel in Sokoto, Nigeria, in 1966 (Causey et al., 1970), through sequential suckling mouse brain passage followed by numerous passages in cell culture. However, disease or lethality in IFN-deficient mice has been reported in studies using several other strains, including Afg-09 2990, Hoti, Turkey-200406546, Ank-2, Ank-15, Ank-16, UAE, Oman-199723179, and Oman-199809166 isolates as well (Oestereich et al., 2014; Hawman et al., 2018; Farzani et al., 2019a; Rodriguez et al., 2019; Spengler et al., 2019); use of these strains, particularly of those from Turkey, in vaccine evaluations is becoming more frequent (Table 7) and represents a key advancement to use of IbAr10200 which has been genetically influenced by its extensive passage history (Lukashev, 2005).

5.2. CCHFV vaccines tested in IFN-deficient mice

CCHFV is lethal in STAT1$^{-/-}$ and IFNAR$^{-/-}$ mice (Table 5). CCHFV vaccines have been tested in all of these strains, with the majority of studies done in IFNAR$^{-/-}$ mice. IFNAR$^{-/-}$ mice infected with the related Dugbe and Hazara orthonairoviruses also develop disease; these viruses have been proposed as surrogate infection models for studying CCHFV (Boyd et al., 2006; Dowall et al., 2012), but have not yet been used to assess protective efficacy of vaccines. To date, detailed below, several vaccine candidates have been evaluated in IFN-deficient mice: plasmid DNA, VLP, plasmid DNA/VLP, VRP, inactivated CCHFV, human adenovirus (Ad) 5, bovine herpesvirus type 4 (BoHV-4), soluble glycoprotein subunits, modified *Vaccinia* virus Ankara (MVA), and mRNA (Tables 6 and 7).

5.2.1. DNA and VLP vaccines—Several CCHFV DNA vaccine studies, either alone or as combination approaches, have been conducted (Table 6). IFNAR$^{-/-}$ mice, and mice

transiently treated at the time of challenge with a monoclonal antibody (MAb-5A3) that blocks signaling via the IFNAR-1 subunit of the murine IFN-α/β receptor, were vaccinated using a prime/boost/boost regimen with a 25 μg dose of a plasmid expressing GPC. GPC is processed to mature structural glycoproteins Gn and Gc and non-structural proteins NSm, GP80/GP160, GP38, and mucin-like domain, which have unknown function. Both IFNAR $^{-/-}$ and immunocompetent mice (pre-antibody block) developed CCHFV-specific antibodies following first vaccination. Prior to challenge, all mice developed highly neutralizing antibodies and both Th1- and Th2-type responses determined by IgG1:IgG2 ratios (Garrison et al., 2017). Despite the robust antibody responses, only 75% of IFNAR$^{-/-}$ mice and 60% of IFN-block mice were protected. Furthermore, antibody responses in survivors to CCHFV-N, a protein not encoded in the vaccine, indicated that the vaccine did not provide sterile immunity.

A DNA vaccine candidate expressing the N protein of CCHFV (pV-N13) provided more promising outcomes. pV-N13, based on the Ank-2 strain isolated from a blood sample of a patient who suffered from hemorrhagic fever in Kastamonu, Turkey, was evaluated alone and with co-delivery of CD24 as a putative adjuvant (Farzani et al., 2019a). Overall, when administered alone or with the pCD24 vector, the N-expressing construct elicited significant cellular and humoral responses in BALB/c mice. IFNAR$^{-/-}$ mice vaccinated with either pV-N13 alone or with CD24 adjuvant were 100% protected, with no significant difference in disease course noted between groups.

Studies by Hinkula et al. also investigated a DNA vaccine encoding a ubiquitin-linked version of CCHFV Gc, Gn, and N, alone and in combination with a new transcriptionally competent virus-like particle (tc-VLP) vaccine (Hinkula et al., 2017). IFNAR$^{-/-}$ mice vaccinated in a prime/boost/boost regimen epidermally/intradermally with DNA (50 μg of each of 3 plasmids) or with tc-VLPs (10^6 IFU) were 100% or 40% protected from lethal IbAr10200 challenge, respectively. A third experimental group given 2 doses of DNA vaccine followed by a single dose of tc-VLPs showed 80% protection.

In addition to efficacy, the immune response was assessed both before and after infection, including analyses of 8 selected Th1 (IFN-γ, TNF-α, IL-12 p70, and IL-2) and Th2 (IL-4, IL-5, IL-10, and GM-CSF) cytokines. Pre-challenge, the group found that a clear difference in the Th1/Th2-type profiles was seen in the 2 types of immunization schedules: induction of Th1-type immunity in DNA-immunized mice and of Th2-type immunity in tc-VLP-immunized mice. Of note, mice that received two doses of DNA vaccine followed by a tc-VLP vaccine boost had a Th1-type profile, similar to the response in mice receiving the DNA vaccine alone. Post-challenge, the researchers were only able to assess Th1/Th2-type profiles in the DNA and DNA with tc-VLP boost groups. Both groups demonstrated a Th1-type response pre-challenge and were later found to develop a Th2-type response post challenge (at 9 dpi). Neutralizing antibodies were detected in all experimental groups, but no clear correlation between protection and titers alone was observed, as titers were highest in the tc-VLP-vaccinated group that had the lowest protective efficacy. Based on these data, the contribution of neutralizing antibodies is unclear, but a Th1-type response was supported as the most effective protective immunity against CCHFV challenge.

5.2.2. VRP vaccines—A VRP vaccine was produced using reverse genetics, with L- and S-segment plasmids based on IbAr10200 and a plasmid expressing the codon-optimized GPC of the Oman-199809166 strain (Scholte et al., 2019). VRPs closely mimic the structure and composition of authentic CCHFV, but the absence of the M segment in VRP particles limits their replication to a single cycle. The VRP vaccine was initially tested with a high (10^5 $TCID_{50}$) or low (10^3 $TCID_{50}$) dose administered in a prime-only vaccination schedule in $IFNAR^{-/-}$ mice. Both anti-N and anti-Gc IgG were detected in mice at 28 days post vaccination, with significantly higher levels in the high-dose group than in the low-dose group. Mice were challenged SC with 100 $TCID_{50}$ of IbAr10200 32 days post vaccination. Although clinical signs were seen in a small subset of vaccinated mice, low-dose vaccination resulted in 80% survival. All mice given the high dose survived with no evidence of clinical signs, providing the first report of a single-dose vaccine conferring complete protection. Additional studies with the VRP platform evaluated efficacy against 2 additional genetically diverse strains of CCHFV, Turkey-200406546 and Oman-199723179 (Spengler et al., 2019). Using the same prime-only vaccination approach (10^5 $TCID_{50}$), mice challenged 28 days post vaccination were completely protected against disease from Oman-199723179 and against death from Turkey-200406546.

5.2.3. Inactivated virus vaccines—All $IFNAR^{-/-}$ mice given a prime/boost/boost vaccination regimen with alum-adjuvanted, chemically inactivated CCHFV rapidly developed CCHFV-specific antibody responses (Canakoglu et al., 2015). Starting 2 weeks after prime inoculation, all $IFNAR^{-/-}$ mice had seroconverted, with the strength of the antibody response corresponding to vaccine dose. Levels of CCHFV-reactive and CCHFV-neutralizing antibodies increased with boost vaccination, with higher titers post boost also correlating to vaccine dose. Following challenge with CCHFV, all groups of mice were partially protected from lethal disease (Table 6). Mice vaccinated with the highest dose (40 μg) controlled viral replication most efficiently, reducing viremia up to 10^4-fold, and had the lowest morbidity (30%) and 80% survival. Mice receiving the intermediate dose (20 μg) controlled viral replication comparably (> 1000-fold reduction in viremia), displayed moderate morbidity (50%), and were equally (80%) protected from lethal disease, whereas those receiving the lowest dose (5 μg) only demonstrated a > 100-fold reduction in viremia, 80% morbidity, and 60% survival.

5.2.4. Subunit vaccines—Kortekaas et al. evaluated CCHFV structural glycoprotein subunit vaccines, produced in insect cells, in $STAT1^{-/-}$ mice using a prime/boost regimen (Kortekaas et al., 2015) (Table 6). One or more modifications to the glycoprotein were incorporated in the subunit design:

1. Gn and Gc ectodomain regions of the GPC were codon-optimized (Gn-e, Gc-e)
2. 17 C-terminal residues were removed from Gc (Gc-eΔ) to increase solubilization
3. A BiP signal sequence was added for improved protein secretion
4. FLAG-tag and Strep-tags were added to facilitate detection and purification, respectively

Following vaccination with the Gc ectodomain, STAT1$^{-/-}$ mice developed significant titers of CCHFV-neutralizing antibodies. However, disease progression and outcome were not significantly different between vaccinated and mock-vaccinated mice challenged IP. In addition, STAT1$^{-/-}$ mice vaccinated with ectodomain subunits of Gn or Gc-eΔ at higher doses than in previous studies seroconverted and developed significant neutralizing antibody titers. Again, however, the vaccinated mice were not protected from weight loss and elevated body temperatures following virus challenge compared to mock-vaccinated mice, even when challenged by the SC route which requires higher doses for lethality.

5.2.5. Attenuated virus vector vaccines—Three virus vector platforms, MVA, Ad-5 and BoHV-4, have been evaluated for CCHFV. The MVA strain of vaccinia virus, derived by serial passaging in chicken cells (Mayr et al., 1975), lost about 15% of the vaccinia virus genome, including genes needed for replicating in human cells, inducing pathogenicity, and blocking the host immune response (Dudek and Knipe, 2006). MVA expressing either N or GPC modified by adding a tPA signal peptide and a C-terminal V5 tag have been constructed and used as vaccine candidates in IFNAR$^{-/-}$ mice. Following a prime/boost vaccine regimen with 10^7 PFU of MVA-GPC, IFNAR$^{-/-}$ mice seroconverted against CCHFV; IFN-γ ELISPOT data indicated that they developed GPC-specific T-cell responses, with 0.5% of total T-cells reactive to GPC. These responses predominantly focused on the NSm, the N-terminus of the Gc, and the mucin-like and the GP38 domains (Buttigieg et al., 2014). Following challenge with CCHFV, IFNAR$^{-/-}$ mice vaccinated with MVA-GPC were fully protected from disease compared to controls (Table 6). IFNAR$^{-/-}$ mice vaccinated with MVA-GPC had lower viremia and tissue viral loads than controls and largely showed no histological abnormalities in the primary target organs (spleen and liver) with absent or minimal viral antigen staining (Buttigieg et al., 2014). Follow-up passive and adoptive transfer studies using the MVA-GPC vaccine indicated that both antibody and T-cell responses contributed to protection, as transfer of both T-cells and antibodies was required for protective effects in these mice (Dowall et al., 2016).

In other experiments, MVA-N was tested in IFNAR$^{-/-}$ mice. Following a prime/boost vaccine regimen with 10^7 PFU of MVA-N, IFNAR$^{-/-}$ mice seroconverted against CCHFV N and developed N-specific T-cell responses as determined by an IFN-γ ELISPOT assay, with 0.1% of total T-cells reactive against N (Dowall et al., 2016). Despite the immunogenicity of the MVA-N vaccine, following CCHFV challenge, both vaccinated and unvaccinated mice developed identical clinical signs and uniformly succumbed to infection at similar rates (Table 6). Furthermore, vaccination with MVA-N did not lower viremia, tissue viral loads, or CCHFV-induced pathological changes to the liver and spleen compared to mock-vaccinated mice (Dowall et al., 2016).

More recently, Zivcec et al. evaluated IFNAR$^{-/-}$ mice vaccinated with an Ad-5-based vaccine expressing the unmodified N (Ad-N) (Zivcec et al., 2018). Mice seroconverted following a single vaccination with 10^7 IFU of Ad-N. Post-challenge, the mice displayed significantly lower levels of viremia, antigen staining, and pathologic changes in the sites of primary viral replication (liver and spleen) than mock-vaccinated animals, but did not have significantly altered tissue virus levels. The mean time to death of Ad-N-vaccinated mice was longer than mock-vaccinated mice (8.5 vs. 5 days), and 33% survived challenge (Table

6). Similarly, IFNAR$^{-/-}$ mice vaccinated and boosted with 10^7 and 10^8 IFU of Ad-N efficiently seroconverted against CCHFV. Following challenge, vaccinated animals displayed significantly lower viremia and tissue viral loads and fewer pathologic changes in the liver and spleen than mock-vaccinated mice. Ad-N prime/boost vaccination increased the protective efficacy to 78% and delayed the mean time to death further (10.5 days) in the animals that succumbed to infection.

Ad-N and DNA (pCDNA3.1 myc/His A, designated as pCD-N1) vaccines were recently compared with a new Bovine herpesvirus type 4 (BoHV-4)-based viral vector vaccine expressing N (BoHV4-ΔTK-CCHFV-N) (Farzani et al., 2019b). In IFNAR$^{-/-}$ mice challenged with lethal doses of the Ank-2 strain, both the BoHV4-ΔTK-CCHFV-N and Ad-N constructs induced 100% protection, although surviving vaccinated mice did exhibit clinical signs starting 1 dpi and persisting for 3–4 days. While protection conferred by Ad-N and BoHV4 vaccines was comparable, potential advantages of the BoHV4 delivery system were supported in T-cell and passive antibody transfer assays; IFNAR$^{-/-}$ mice given splenocytes and serum from BALB/c mice immunized with BoHV4-ΔTK-CCHFV-N had higher post-challenge survival rates (75%) compared to mice given cells and serum from BALB/c mice immunized with pCD-N1 (50%) or Ad-N (50%).

5.2.6. VSV vaccines—To produce infectious VSV with CCHFV-GPC, Rodriguez et al. used a modified method, utilizing in trans VSV glycoprotein (G) complementation, to generate the replication-competent ΔGrVSV-CCHFV-GPCΔ vaccine candidate based on the IbAr10200 sequence (Rodriguez et al., 2019). STAT1$^{-/-}$ mice were vaccinated IP in a prime or prime/boost strategy and challenged IP with a target dose of 100 PFU of IbAr10200 or Turkey-200406546 strains. Conferring any level of protection depended on a replication-competent VSV vaccine; STAT1$^{-/-}$ mice vaccinated with replication-deficient VSV (10^6 PFU), with or without boost (with replication-deficient or competent VSV at the same dose) and challenged with IbAr10200 were not protected from disease or death. Efficacy improved in a dose-dependent manner with administration of replication-competent VSV, and a single 10^6 PFU dose of this vaccine partially protected mice from death; a 10^2 PFU dose failed to protect any of the mice. In contrast, when mice given the same replication-competent vaccine were challenged with Turkey-200406546, a single 10^7 PFU dose protected against death (not disease), and a prime/boost regimen conferred complete protection against both death and disease.

5.2.7. mRNA vaccines—A conventional, non-replicating naked mRNA-based construct expressing the non-optimized S segment of the Ank-2 strain was generated for immunogenicity studies in C57BL/6 and challenge studies in IFNAR$^{-/-}$ mice (Farzani et al., 2019c). Mice were immunized in- tramuscularly with mRNA (25 μg) by prime or prime/boost. C57BL/6 and IFNAR$^{-/-}$ mice developed anti-N specific immune responses prior to challenge, as demonstrated by anti-N IgG analyses. IFNAR$^{-/-}$ mice were challenged with 100 LD_{50} (1000 $TCID_{50}$) of Ank-2 at 4 weeks (prime only) or 2 weeks (prime/boost) following the last vaccination. Prime/boost vaccination conferred 100% protection, whereas the prime dose alone only protected 50% of mice. Despite increased survival in vaccinated

mice, both prime and prime/boost mice became ill, in a manner similar to unvaccinated controls during the first week post challenge, prior to recovering.

6. Phenuiviruses

6.1. Susceptibility of IFN-deficient mice to RVFV

Immunocompetent mice (e.g., BALB/c, C57BL/6J, 129/Sv) are extremely sensitive to infection with wild-type RVFV (e.g., ZH598 and ZH501); IP or SC inoculation with as little as 10 PFU causes acute hepatitis and death of the animals within a few days (Vialat et al., 2000). As a result, there has been little need to assess vaccines in IFN-deficient mice.

Attenuation of RVFV for immunocompetent mice requires disruption of virus-mediated IFN antagonism. IFN-deficient mice have largely been used in studies with 2 strains of RVFV, MP-12 and clone 13, that have defects in the IFN antagonist NSs gene. MP12 carries attenuating mutations in each genomic segment, including the NSs-encoding small (S) segment, and clone 13 has a defective NSs gene with a large inframe deletion. Both strains were attenuated in IFN-γ receptor-deficient mice but highly virulent in IFNAR$^{-/-}$ mice (Bouloy et al., 2001; Lorenzo et al., 2010). When MP-12 was administered intranasally at a dose of 1.58×10^6 $TCID_{50}$ in immunocompetent 129S1/SvlmJ and 129S6/SvEv mice and IFN-deficient STAT1$^{-/-}$ mice, weight loss and clinical signs were reported in all strains, but disease was most severe in STAT1$^{-/-}$ mice; weight loss began at 2 dpi followed by decreased activity, huddling, hunched posture, and ruffled fur as early as 4 dpi. Infection in STAT1$^{-/-}$ was partially lethal, with end-point criteria reached ~8–10 dpi and associated with severe weight loss (~25%) and neurological abnormalities, such as cage circling and head pressing (Lang et al., 2016).

6.2. Vaccines tested in IFN-deficient mice infected with RVFV

RVFV vaccine testing in IFN-deficient mice has been limited to IFNAR$^{-/-}$ mice using plasmid DNA or non-replicating MVA platforms. RVFV vaccines are predominantly based on antigens located on the medium (M) and S viral segments. The M segment encodes the structural Gn and Gc attachment and entry glycoproteins and the non-structural IFN antagonist NSm, while the S segment encodes the N and the non-structural NSs.

6.2.1. DNA vaccines—Both S and M segment-derived DNA vaccines have been evaluated for RVFV independently and in combination (Table 8). Vaccination with NP alone did not generate a neutralizing antibody response and mice were only partially protected from disease (43% survival) (Lorenzo et al., 2010). Subsequent studies evaluated fusing additional proteins to NP to increase immunogenicity and protective efficacy. Following a prime/boost vaccination regimen with 100 μg of plasmid DNA, all IFNAR$^{-/-}$ mice vaccinated with an NP-expressing plasmid seroconverted against NP. Mice vaccinated with fused NP plasmids had higher titers of NP-specific antibodies than wild-type NP; NP fused to ubiquitin generated the highest titers of NP-specific antibodies, followed by that to CD154, the 3 C3d domains, and finally the lysosome integral membrane protein II (LIMPII) (Boshra et al., 2011). Corresponding to the antibody data, 71% of IFNAR$^{-/-}$ mice vaccinated with a plasmid expressing NP fused to ubiquitin were protected from lethal

disease. NP tethered to CD154 protected 43%, and NP tethered to 3 C3d, 29%; both NP fused to LIMPII and wild-type NP protected 14% of the animals (Table 8) (Boshra et al., 2011).

Two types of RVFV M-segment DNA plasmid vaccines have been evaluated: M1, which encodes the complete M coding region including NSm, Gn, and Gc; and M4, which only encodes the structural viral glycoproteins Gn and Gc (Lorenzo et al., 2010). IFNAR$^{-/-}$ mice vaccinated with M1 neither mounted a neutralizing response nor were protected from lethal disease, and the addition of NP in combination did not elicit responses specific to NP or the glycoproteins, nor improved protection. However, mice given a prime/boost vaccination regimen of 100 μg of M4 alone developed neutralizing antibody responses and were fully protected from disease. Interestingly, when NP was given in combination with M4, only 57% of the mice were protected. Different M4:N ratios were tested to further characterize contributors to this decreased protection, and confirmed that the protective effect was dependent on the M4 dose. Protective efficacy decreased to 43% and 29% as the amount of M4 in the combination was reduced to 40 μg and 20 μg, respectively, demonstrating the importance of antibody responses to Gn and Gc in these mice. Altogether, these studies suggest that in DNA vaccination strategies, antibody levels correlate to protection of IFNAR$^{-/-}$ mice.

6.2.2. MVA vaccines—An MVA vaccine that expresses Gn and Gc (MVA-GnGc) was developed for RVFV and was fully protective in BALB/c mice (Lopez-Gil et al., 2013). However, only 14% of IFNAR$^{-/-}$ mice vaccinated with a single dose of 10^7 PFU of MVA-GnGc were protected from lethal challenge (Table 8). This is in contrast to the aforementioned studies by the same group, which showed that the DNA vaccine pCMV-M4 protected these mice (Lorenzo et al., 2010), suggesting that delivery method can greatly influence vaccine efficacy.

7. Conclusions

A disadvantage of many studies testing HF vaccines in mice has been that the challenge virus may have been adapted to mice, and therefore may have substantial genetic differences from the virus that causes human disease. The use of IFN-deficient mice is not limited by availability of mouse-adapted strains; they can be used to test vaccines against a virus isolated from a patient and minimally passaged in cell culture, including new emergent strains. Furthermore, creating mouse-adapted variants of certain viruses (e.g., CCHFV) has not been possible; vaccine evaluation in small animal models for these viruses relies solely on immunodeficient mice. While using IFN-deficient mice has certain advantages, the limitations of these mice should be considered, particularly in vaccine studies. For example, some live attenuated vaccines cause disease in IFN-deficient mice, limiting their use in vaccine evaluation; this is especially a problem with RVFV and with using unmodified viral vector vaccines like VSV.

If a candidate vaccine protects IFN-deficient mice against a virus isolated from a human patient, it is an encouraging sign that the same vaccine will provide protection in other immunocompetent laboratory animals and in humans. In contrast, a vaccine that fails to

protect IFN-deficient mice may not produce similar results in immunocompetent animals or humans. As discussed in the companion paper, defects in the innate antiviral responses in IFN-deficient mice may hinder adaptive immune responses to vaccination in many ways; no consistent effect on adaptive immunity across viruses or vaccine platforms can be accounted for in data interpretation without the inclusion of appropriate wild-type control mice. Also, if mice lacking type I or type I/type II IFN responses are particularly susceptible to a challenge virus, a vaccine failing to protect these mice may otherwise be effective in immunocompetent animals or humans.

At the moment, lack of data prevents correlating outcomes of vaccination in IFN-deficient mice and results obtained in other laboratory animals or humans. Only the VSV-Ebola vaccine, which successfully protected IFNAR$^{-/-}$ mice, has been evaluated in non-human primates and tested for human use. Assessing the value of IFN-deficient mice for vaccine research must await reports of further animal testing. At this time, evaluation in IFN-deficient mice in conjunction with other immunocompetent models (when available) remains a viable approach to preliminary assessment of HF vaccine candidates.

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Table 1

Overview of vaccine platforms tested in IFN-deficient mice.

Vaccine platform	Advantages	Disadvantages
Attenuated virus	• Display native proteins • Ease of production • Single vaccination alone often sufficient for protection • Long duration of immunity • Induces both cellular and humoral immunity	• Safety concerns (use in pregnant or immunocompromised persons; potential for spread of the vaccine virus in the population; etc.) • Instability (correct storage/transport conditions to maintain infectivity)
Inactivated virus	• Safety (non-replicating) • Acceptable platform already widely in use	• Limited duration of immunity • May requires multiple doses • Production requires containment • Denaturation of antigens during inactivation
Subunit	• Safety (contain only the antigenic parts of the pathogen) • Ease of production (no requirement for inactivation or high containment) • Established platform • Stability (depending on format, i.e., purified antigen)	• Limited duration of immunity • Decreased efficacy • May require multiple doses
DNA	• Safety (do not contain live components, do not integrate into host genome) • Ease of production (no requirement for inactivation or high containment) • Similarity to native antigens • Stability (DNA > RNA)	• May requires multiple doses • Not yet an accepted platform for human use
Non-replicating viral vectors	• Display native proteins • Ease of production (no requirement for inactivation or high containment) • Some are now licensed for human use	• May require multiple doses • Laborious to produce in high quantities

Table 2

Reports of severe or fatal disease in filovirus-infected IFN-deficient mice.

Virus	Strain	Knockout	Outcome	References
EBOV	Kikwit	STAT1$^{-/-}$ (129S6/SvEv)	L	(Raymond et al., 2011; Zumbrun et al., 2012)
		IFNAR$^{-/-}$ (C57BL/6)	PL	Brannan et al. (2015)
		IFN γR$^{-/-}$ (C57BL/6)	L	Zumbrun et al. (2012)
		IFN α/β/γR$^{-/-}$	L	Comer et al. (2019)
	Mayinga	STAT1$^{-/-}$ (129S6/SvEv)	L	Raymond et al. (2011)
		STAT1$^{-/-}$ (129)	L	Bray (2001)
		IFNAR$^{-/-}$ (129)	L	Bray (2001)
		WTIFNAR$^{-/-}$ (C57BL/6)	PL	Lüdtke et al. (2017)
		IFNAR$^{-/-}$ IFNAR$^{-/-}$ (C57BL/6)	L	Lüdtke et al. (2017)
	Makona	IFNAR$^{-/-}$	PL	Smither et al. (2016)
	Yambuku-Ecran (E718)	IFNAR$^{-/-}$	L	Lever et al. (2012)
SUDV	Boniface	IFNAR$^{-/-}$ (129)	L	Bray (2001)
		IFNAR$^{-/-}$ (C57BL/6)	PL	Brannan et al. (2015)
		IFNAR$^{-/-}$ (C57BL/6)	L	(Frei et al., 2016; Froude et al., 2018)
	Gulu	IFNAR$^{-/-}$ (C57BL/6)	PL	Brannan et al. (2015)
		IFN α/β/γR$^{-/-}$	PL	Comer et al. (2019)
MARV	Angola	IFN α/β/γR$^{-/-}$	L	Comer et al. (2019)
	Musoke	IFNAR$^{-/-}$ (129)	PL	Bray (2001)
	Poppinga	IFNAR$^{-/-}$	L	Lever et al. (2012)
RAVV	WT	STAT1$^{-/-}$ (129S6/SvEv)	PL/L[a]	Raymond et al. (2011)

Studies with wild-type (non-rodent-adapted) viruses that cause severe or fatal disease in mice.

[a]Serially euthanized or succumbed to disease by 5 days post infection; L, lethal (100% mortality achieved with at least one virus dose and inoculation route combination); PL, partially lethal (< 100% mortality at all doses and by all inoculation routes attempted in study); RAVV, Ravn virus; WT, wild-type.

Table 3

Filovirus vaccines

Knockout	Vaccine	Antigen	Vaccine virus/strain	Schedule	Dose/delivery route	Challenge post-vax[a]	Challenge virus/strain	Challenge dose/route	Efficacy (survival)	Ref.
STAT1$^{-/-}$	EBOVΔVP30	Whole virus	EBOV/ Mayinga	P	10^6 FFU/IP	NA	NA	NA	NA	Halfmann et al. (2009)
	VLP	GP, NP, VP40	EBOV/ Mayinga	P/B/B, 3-wk interval	10 μg/IM	40 d	EBOV/MA-Mayinga	1000 PFU/IP	0%	Raymond et al. (2011)
IFNAR$^{-/-}$	VEE VRP-EBOV GP	GP	EBOV/ Mayinga	P	10^6–10^7 IU/SC	4 wk	EBOV/Kikwit or SUDV/ Boniface	1000 PFU/IP	90–100%	Brannan et al. (2015)
	rVSVΔG/EBOV GP	GP	EBOV/ Mayinga	P/B, 3-wk interval	2×10^7 SRIP/IM	7 wk	EBOV/ Mayinga	1000 PFU/IP	Protective[b]	Lennemann et al. (2017)
		GP 7G	EBOV/ Mayinga	P/B, 3-wk interval	2×10^7 SRIP/IM	7 wk	EBOV/ Mayinga	1000 PFU/IP	Protective[b]	Lennemann et al. (2017)
		GP 7G	EBOV/ Mayinga	P/B, 3-wk interval	2×10^7 SRIP/IM	7 wk	SUDV/ Boniface	1000 PFU/IP	Not protective[b]	Lennemann et al. (2017)
		GP 7Gm8G	EBOV/ Mayinga	P/B, 3-wk interval	2×10^7 SRIP/IM	7 wk	EBOV/ Mayinga	1000 PFU/IP	Protective[b]	Lennemann et al. (2017)
		GP 7Gm8G	EBOV/ Mayinga	P/B, 3-wk interval	2×10^7 SRIP/IM	7 wk	SUDV/ Boniface	1000 PFU/IP	Not protective[b]	Lennemann et al. (2017)
	rVSVΔG/SUDV GP	GP	SUDV/ Boniface	P/B, 3-wk interval	2×10^7 SRIP/IM	7 wk	SUDV/ Boniface	1000 PFU/IP	Protective[b]	Lennemann et al. (2017)

None of the studies reported use of adjuvant.

B, boost; FFU, focus-forming units; IM, intramuscular; IP, intraperitoneal; IU, infectious units; MA, mouse-adapted; NA, not applicable; P, prime; PFU, plaque-forming units; SC, subcutaneous; 7G, sequence was modified to eliminate the 7 N-linked glycans in the core region (receptor binding domain and glycan cap) of GP1; 7Gm8G, sequence was modified to eliminate all 15 N-linked glycans in GP1; rVSV, recombinant vesicular stomatitis virus; SRIP, single-round infectious particles.

[a]time since last vaccine dose administered.

[b]Protective efficacy based on weight loss, reported as positive or negative effect.

Table 4

Reports of severe or fatal disease in arenavirus-infected IFN-deficient mice.

Virus	Strain	Knockout (background)	Outcome	References
LASV	rJosiah	STAT1$^{-/-}$ (129S6/SvEv)	L	Yun et al. (2013)
	LF2384-NS-DIA-1	STAT1$^{-/-}$ (129S6/SvEv)	L	Yun et al. (2013)
		STAT1$^{-/-}$ (129S6/SvEv)	PL	Yun et al. (2015)
	LV2350-NS-DIA-1	STAT1$^{-/-}$ (129S6/SvEv)	PL	Yun et al. (2015)
	BA366	IFNAR$^{-/-\ B6}$ (C57BL/6)[a]	L	Oestereich et al. (2016)
		B6 $^{IFNAR-/-}$ (C57BL/6)[a]	PL	Oestereich et al. (2016)
		IFNAR$^{-/-\ IFNAR-/-}$ (C57BL/6)[a]	PL	Oestereich et al. (2016)
MACV	Carvallo	STAT1$^{-/-}$ (129S6/SvEv)	L	Bradfute et al. (2011)
		IFN α/β/γR$^{-/-}$ (129)	L	(Koma et al., 2016, 2015)
JUNV	Romero	IFN α/β/γR$^{-/-}$ (129/SvPAS)	L	Kolokoltsova et al. (2010)
TCRV	TRVL 11573	IFN α/β/γR$^{-/-}$ (129/SvPAS)	L	Gowen et al. (2010)

Studies reporting severe (PL, partially lethal; < 100% mortality at all doses and by all inoculation routes attempted in study) or fatal disease (L, lethal; 100% mortality achieved with at least one virus dose and inoculation route combination).

TCRV, Tacaribe virus.

[a]Chimeric mice generated by transplantation of wild-type bone marrow cells into irradiated mice (indicated as background with transplanted bone marrow origin in superscript).

Table 5

Reports of severe or fatal disease in CCHFV-infected IFN-deficient mice.

CCHFV Strain	Knockout (background)	Outcome	References
IbAr10200	STAT1$^{-/-}$ (129S6/SvEv)	L	Bente et al. (2010)
	IFNAR$^{-/-}$ (129Sv/Ev)	L	Dowall et al. (2016)
	IFNAR$^{-/-}$ (129 Sv/Ev)	L	Bereczky et al. (2010)
	IFNAR$^{-/-}$ (C57BL/6)	L	Zivcec et al. (2013)
Afg-09 2990	IFNAR$^{-/-}$ (129Sv)	L	Oestereich et al. (2014)
Hoti	IFNAR$^{-/-}$ (C57BL/6)	L	Hawman et al. (2018)
Turkey-200406546	IFNAR$^{-/-}$ (C57BL/6)	L	Spengler et al. (2019)
Turkey-200406546	STAT1$^{-/-}$ (129S6/SvEv)	L	Rodriguez et al. (2019)
Ank-2	IFNAR$^{-/-}$ (129S7/SvEvBrd)	L	Farzani et al. (2019a)
Ank-15	IFNAR$^{-/-}$ (129S7/SvEvBrd)	L	Farzani et al. (2019a)
Ank-16	IFNAR$^{-/-}$ (129S7/SvEvBrd)	L	Farzani et al. (2019a)
UAE	IFNAR$^{-/-}$ (C57BL/6)	PL	Spengler et al. (2019)
Oman-199723179	IFNAR$^{-/-}$ (C57BL/6)	PL	Spengler et al. (2019)

Studies reporting severe (PL, partially lethal; < 100% mortality at all doses and by all inoculation routes attempted in study) or fatal disease (L, lethal; 100% mortality achieved with at least one virus dose and inoculation route combination) in IFN-deficient mice.

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Table 6

CCHFV vaccines evaluated against IbAr10200 strain.

Knockout	Vaccine	Antigen	Vaccine virus	Schedule	Dose/route; adjuvant (if applicable)	Challenge post-vax[a]	Challenge dose/ route	Efficacy (survival)	Ref.
IFNAR$^{-/-}$	Plasmid DNA	N*, Gn*, Gc*	IbAr10200	P/B/B, 4 wk, 3 wk interval	50 μg/ID, EP	6 wk	400 FFU/IP	100%	Hinkula et al. (2017)
	Plasmid DNA & VLP	L, N*, Gn*, Gc*, GPC*	IbAr10200	P/B/B, 4 wk, 3 wk interval	50 μg DNA, 10^6 VLP/ID, EP, then IP	6 wk	400 FFU/IP	80%	Hinkula et al. (2017)
	VLP	L, N, GPC	IbAr10200	P/B/B, 4 wk, 3 wk interval	10^6 VLP/IP	6 wk	400 FFU/IP	40%	Hinkula et al. (2017)
	VRP	L, N, GPC	IbAr10200 N, L + Oman GPC	P	10^3 $TCID_{50}$ (low dose), or 10^5 $TCID_{50}$ (high dose)/SC	4 wk	100 TCID50/SC	80 (low) – 100% (high)	(Scholte et al., 2019) (Spengler et al., 2019)
	Plasmid DNA	GPC	IbAr10200	P/B/B, 3 wk intervals	25 μg/IM, EP	4 wk	100 PFU/IP	71%	Garrison et al. (2017)
	MVA	GPC†	IbAr10200	P/B, 2 wk interval	10^7 PFU/IM	2 wk	200 $TCID_{50}$/ID	100%	(Buttigieg et al., 2014; Dowall et al., 2016b)
		N†	IbAr10200	P/B, 2 wk interval	10^7 PFU/IM	2 wk	200 TCID50/ID	0%	Dowall et al. (2016a)
	Human Ad 5	N	IbAr10200	P	10^7 IFU/IM	4 wk	1000 LD_{50}/ID	33%	Zivcec et al. (2018)
		N	IbAr10200	P/B, 4 wk interval	10^7 IFU P, 10^8 IFU B/IM P, IN B	4 wk	1000LD_{50}/ID	78%	Zivcec et al. (2018)
STAT1$^{-/-}$	Subunit	Gn-e	IbAr10200	P/B, 3 wk interval	15 μg/IP; Sigma adjuvant system	2 wk	100 PFU/SC	0%	Kortekaas et al. (2015)
		Gc-eΔ	IbAr10200	P/B, 3 wk interval	7.5 μg/IP; Sigma adjuvant system	2 wk	100 PFU/SC	0%	Kortekaas et al. (2015)
		Gc-e	IbAr10200	P/B, 3 wk interval	1.4 μg/IP; Sigma adjuvant system	2 wk	100 PFU/IP	0%	Kortekaas et al. (2015)
	rVSV (replication deficient)	GPC	IbAr10200	P or P/B (2 wk interval)	10^6 PFU/IP	3 wk	100 PFU/IP	0%	Rodriguez et al. (2019)
	rVSV (replication competent)	GPC	IbAr10200	P	10^2 or 10^6 PFU/IP	3 wk	100 PFU/IP	0% (low) – 40% (high)	Rodriguez et al. (2019)
WT + IFN block	Plasmid DNA	GPC	IbAr10200	P/B/B, 3 wk intervals	25 μg/IM, EP	4 wk	100 PFU/IP	60%	Garrison et al. (2017)

[a] refers to time since last vaccine dose administered. Sequence was modified by:

*, addition of ubiquitin to the target antigen;

†, addition of tPA signal peptide and C-terminal V5 tag; -e, addition of BiP signal sequence, C-terminal Flag tag, and 3 Strep tags separated by glycine linker regions; or -eΔ, removal of terminal 17 residues and addition of BiP signal sequence, C-terminal Flag tag, and 3 Strep tags separated by glycine linker regions. Ad, adenovirus; B, boost; EP, electroporation; FFU, focus-forming units; ID, intradermal (between shoulders); IFN, interferon; IFU, infectious units; IM, intramuscular; IN, intranasal; IP, intraperitoneal; LD_{50}, 50% lethal dose in $IFNAR^{-/-}$ mouse; MVA, modified *Vaccinia* Ankara; P, prime; SC, subcutaneous; PPFU, pseudo plaque-forming units; PFU, plaque-forming units; rVSV, recombinant vesicular stomatitis virus; $TCID_{50}$, tissue culture infectious dose 50%; VRP, viral replicon particle; WT, wild-type.

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Table 7

CCHFV vaccines evaluated against strains other than IbAr10200.

Knockout	Vaccine	Antigen	Vaccine virus	Schedule	Dose/route; adjuvant (if applicable)	Challenge post-vax[a]	Challenge strain	Challenge dose/route	Efficacy (survival)	Ref.
IFNAR$^{-/-}$	Plasmid DNA	N	Ank-2	P/B, 2 wk interval	50 μg DNA IM	2 wk	Ank-2	1000 $TCID_{50}$/IP	100%	Farzani et al. (2019a)
		N	Ank-2	P/B, 2 wk interval	40 μg DNA IM + 10 μg CD24 IM	2 wk	Ank-2	1000 $TCID_{50}$/IP	100%	Farzani et al. (2019a)
	VRP	L, N, GPC	IbAr10200 N, L + Oman GPC	P	10^5 $TCID_{50}$/SC	4 wk	Turkey-200406546	100 $TCID_{50}$/SC	100%	Spengler et al. (2019)
		L, N, GPC	IbAr10200 N, L + Oman GPC	P	10^5 $TCID_{50}$/SC	4 wk	Oman-199723179	100 $TCID_{50}$/SC	100%	Spengler et al. (2019)
	Inactivated, purified virus pellet	L, N, GPC	Turkey-Kelkit06	P/B/B, 3 wk intervals	20, or 40 μg/IP; alum	2 wk	Turkey-Kelkit06	1000 PPFU/IP	80%	Canakoglu et al. (2015)
		L, N, GPC	Turkey-Kelkit06	P/B/B, 3 wk intervals	5 μg/IP; alum	2 wk	Turkey-Kelkit06	1000 PPFU/IP	60%	Canakoglu et al. (2015)
	BoHV-4	N	Ank-2	P/B, 2 wk interval	10^2 $TCID_{50}$ IP	2 wk	Ank-2	1000 TCID50/IP	100%	Farzani et al. (2019b)
	mRNA	N	Ank-2	P	25 μg IM	4 wk	Ank-2	1000 $TCID_{50}$/IP	50%	Farzani et al. (2019c)
		N	Ank-2	P/B, 2 wk interval	25 μg IM each dose	2 wk	Ank-2	1000 TCID50/IP	100%	Farzani et al. (2019c)
STAT1$^{-/-}$	rVSV (replication competent)	GPC	IbAr10200	P or P/B (2 wk interval)	10^7 PFU/IP	3 wk	Turkey-200406546	50 PFU/IP	100% (P/B only)	Rodriguez et al. (2019)

[a] refers to time since last vaccine dose administered.; B, boost; BoHV-4, bovine herpesvirus type 4; FFU, focus-forming units; ID, intradermal (between shoulders); IFN, interferon; IFU, infectious units; IM, intramuscular; IN, intranasal; IP, intraperitoneal; LD_{50}, 50% lethal dose in IFNAR$^{-/-}$ mouse; P, prime; SC, subcutaneous; PPFU, pseudo plaque-forming units; PFU, plaque-forming units; rVSV, recombinant vesicular stomatitis virus; $TCID_{50}$, tissue culture infectious dose 50%; VRP, viral replicon particle; WT, wild-type.

Table 8

RVFV vaccines tested in IFNAR$^{-/-}$ mice.

Vaccine	Antigen	Schedule	Dose/delivery	Challenge post-vax[a]	Challenge virus/ strain	Challenge dose/route	Efficacy (survival)	Ref.
Plasmid DNA	N, Gn, Gc, NSm	P/B, 2 wk interval	100 μg/IM	15 d	MP-12	2×10^4 PFU/IP	0%	Lorenzo et al. (2010)
	Gn, Gc, NSm	P/B, 2 wk interval	100 μg/IM	15 d	MP-12	2×10^4 PFU/IP	0%	Lorenzo et al. (2010)
	N, Gn, Gc	P/B, 2 wk interval	20–100 μg/IM	15 d	MP-12	2×10^4 PFU/IP	29–57%	Lorenzo et al. (2010)
	Gn, Gc	P/B, 2 wk interval	1–100 μg/IM	15 d	MP-12	2×10^4 PFU/IP	0–100%, full protection achieved at 100 μg dose	(Lorenzo et al., 2010; Boshra et al., 2011)
	N	P/B, 2 wk interval	100 μg/IM	15 d	MP-12	2×10^4 PFU/IP	14–43%	(Lorenzo et al., 2010; Boshra et al., 2011)
	N[#]	P/B, 2 wk interval	100 μg/IM	15 d	MP-12	2×10^4 PFU/IP	33%	Boshra et al. (2011)
	N[%]	P/B, 2 wk interval	100 μg/IM	15 d	MP-12	2×10^4 PFU/IP	43%	Boshra et al. (2011)
	N[*]	P/B, 2 wk interval	100 μg/IM	15 d	MP-12	2×10^4 PFU/IP	71%	Boshra et al. (2011)
	N[&]	P/B, 2 wk interval	100 μg/IM	15 d	MP-12	2×10^4 PFU/IP	14%	Boshra et al. (2011)
MVA	Gn[$], Gc[$]	P	10^7 PFU/IP	15 d	RVFV isolate 56/74	1000 PFU/IP	14%	Lopez-Gil et al. (2013)

None of the studies reported use of adjuvant.

All vaccines were based on RVFV MP-12 strain. In all studies, virus challenge was given 15 days post vaccination. Sequence was modified by addition of:

[#], 3 tandem repetitions of the C3d gene joined by short linker sequences to the antigen N-terminus;

[%], CD154 and a flexible linker to the antigen N-terminus;

[*], ubiquitin to the antigen N-terminus;

[&], 20 amino acid from the C-terminal tail of LIMPII to the antigen C-terminus; or

[$], tPA signal peptide and C-terminal V5 tag. B, boost; IM, intramuscular; IP, intraperitoneal; MVA, modified *Vaccinia* Ankara; P, prime; PFU, plaque-forming units.

[a]Refers to time since last vaccine dose administered.