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**Supplementary Fig 1. Survival of suckling mice inoculated with CCHFV or VRP.** One- or two-day-old CD-1 suckling mice (Charles River; 022CD1) were inoculated intracranially with 1.31 × 102 TCID50 of recombinant IbAR10200 CCHFV (n = 25) or ~100-fold higher dose of CCHFV VRP (1.26 × 104 TCID50; n =11), and monitored daily for clinical signs. Suckling mice with abnormal development prior to inoculation or documented failure to thrive were omitted from analysis (n = 2, CCHFV; n = 1, VRP). All suckling mice inoculated with CCHFV succumbed to acute onset disease by 7 dpi. No clinical signs were observed in VRP-inoculated mice; all VRP inoculated mice survived until study completion (19 dpi).



**Supplementary Fig 2. Clinical scores in VRP-vaccinated IFNAR-/- mice after heterologous CCHFV challenge.** Mice were challenged SC with a target dose of 100 TCID50 of indicated CCHFV strains 28 days after VRP vaccination. Mice were scored based on 14 parameters: 2 points each for quiet, dull, responsive (QDR) disposition, hunched back, or ruffled coat; 3 points each for dehydration or abnormal huddling/hypoactivity; 5 points each for presence of neurological signs (ataxia, circling, tremors, or paresis), abnormal breathing, or anemia; 7 points for weight loss of >20% from baseline (-1 dpi); 10 points each for inability to bear weight, paralysis, frank hemorrhage/bleeding, moribund state, or weight loss of >25% from baseline. Animals were humanely euthanized when end-point criteria were reached (clinical score ≥ 10), or at study completion (21 days post challenge).

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**Supplementary Fig 3. Post-challenge RT-PCR analysis of blood and tissues.** RT-PCR analyses of viral RNA (S segment) in blood and tissues from mice challenged with a target dose of 1 × 102 TCID50 of indicated CCHFV strains 28 days after VRP vaccination. Tissues were collected when mice reached end-point criteria (open symbols) or at completion of the study (closed symbols; 21 days post challenge).

**Supplementary Table 1.** Serological analyses of CCHFV-infected IFNAR-/- mice.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| CCHFV strain | | DPI | Outcome | Anti-NP IgM | Anti-NP IgG | Anti-Gc IgG |
| IbAr10200 | 1 | 5 | Fatal | 0 | 23 | 154 |
| 2 | 5 | Fatal | 0 | 1 | 29 |
| 3 | 5 | Fatal | 5 | 15 | 123 |
| 4 | 5 | Fatal | 0 | 7 | 55 |
| 5† | 5 | Fatal | NS | NS | NS |
| Turkey | 1 | 5 | Fatal | 18 | 4 | 50 |
| 2 | 7 | Fatal | 1690 | 2932 | 2985 |
| 3‡ | 7 | Fatal | NS | 6115 | NS |
| 4 | 7 | Fatal | 1976 | 7555 | 6011 |
| 5 | 6 | Fatal | 165 | 148 | 214 |
| UAE | 1 | 21 | Survivor | 183 | 38093 | 1740 |
| 2 | 21 | Survivor | 1441 | 39895 | 2302 |
| 3 | 7 | Fatal | 6026 | 6090 | 15722 |
| 4† | 7 | Fatal | NS | NS | NS |
| 5 | 7 | Fatal | 4924 | 1840 | 2508 |
| Oman-97 | 1 | 21 | Survivor | 352 | 19111 | 2207 |
| 2 | 21 | Survivor | 105 | 19578 | 2469 |
| 3 | 21 | Survivor | 85 | 38597 | 1950 |
| 4 | 21 | Survivor | 518 | 64279 | 3065 |
| 5 | 7 | Fatal | 5428 | 11229 | 16193 |
| Oman-98 | 1 | 21 | Survivor | 215 | 41492 | 3880 |
| 2 | 21 | Survivor | 413 | 100248 | 4487 |
| 3 | 21 | Survivor | 46 | 36379 | 1266 |
| 4 | 21 | Survivor | 40 | 36408 | 1773 |
| 5 | 21 | Survivor | 267 | 21201 | 2253 |

IgM and IgG antibodies against CCHFV NP and IgG against CCHFV Gc in plasma were obtained at disease end-point or at completion of study at 21 dpi. Values presented in antibody activity units (AAU). All animals were challenged subcutaneously with 1 × 102 TCID50 ofthe indicated CCHFV strains. †No plasma sample obtained. ‡Not enough sample to run all assays. NS, no sample.

**Supplementary Table 2.** Primer and hydrolysis probe sequences for CCHFV strain-specific S segment (NP) RT-PCR.

|  |  |  |
| --- | --- | --- |
| **CCHFV Strain** |  | **Sequence (5′– 3′)** |
| **IbAr10200** | Fwd | ATGAACAGGTGGTTTGAAGAGTT |
| Rev | TGGCACTGGCCATCTGA |
| Probe | 6FAM/TGTCCAAAT/ZEN/TGGGAACACTCTCGCA/IABKFQ |
| **Oman\*** | Fwd | TGATGATGCTGCCTTAGGATC |
| Rev | TGGAGACTGTTACCAACAAGA |
| Probe | 6FAM/TGCAGCAGG/ZEN/TGCTCAGAGGCTACA/IABKFQ |
| **Turkey** | Fwd | GGCTGAGTGTGGAGCACC |
| Rev | AACAGGATTTAACATACAGGACATG |
| Probe | 6FAM/TCCCTTGTT/ZEN/GGCAAGCAGTCTCCA/IABKFQ |
| **UAE** | Fwd | ATGGAGACTGTTACCAACAAG |
| Rev | TGATGATGCTGCCTTAGGATC |
| Probe | 6FAM/TGCAGCAGG/ZEN/TGCTCAGAGGCTACA/IABKFQ |

\*Primer-probe set detects both Oman-97 and Oman-98. Fwd, forward primer; Rev, reverse primer.

**Supplementary Table 3.** Serology and associated outcome post challenge in CCHFV VRP-vaccinated IFNAR-/- mice.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Vaccine status + challenge strain | | DPI | Outcome | Anti-NP IgM | | Anti-NP IgG | | Anti-Gc IgG | |
| **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** |
| VRP + IbAr10200 | 1 | 21 | Survivor | 0 | 0 | 3174 | 10441 | 23 | 90 |
| 2 | 21 | Survivor | 4 | 6 | 4291 | 9375 | 14 | 63 |
| 3 | 21 | Survivor | 122 | 537 | 5363 | 5523 | 114 | 362 |
| VRP + Oman-97 | 1 | 21 | Survivor | 0 | 162 | 6045 | 135840 | 16 | 525 |
| 2 | 21 | Survivor | 109 | 287 | 11946 | 26918 | 44 | 129 |
| 3 | 21 | Survivor | 55 | 89 | 4154 | 16563 | 52 | 52 |
| 4 | 21 | Survivor | 7 | 104 | 4915 | 28952 | 218 | 503 |
| 5 | 21 | Survivor | 71 | 194 | 3998 | 8575 | 67 | 193 |
| 6 | 21 | Survivor | 11 | 8 | 9956 | 34684 | 31 | 136 |
| VRP + Turkey | 1 | 21 | Survivor | 0 | 2 | 4633 | 24000 | 17 | 39 |
| 2 | 21 | Survivor | 4 | 8 | 3526 | 6313 | 73 | 111 |
| 3 | 21 | Survivor | 7 | 4 | 6591 | 29946 | 24 | 126 |
| 4 | 21 | Survivor | 0 | 19 | 5384 | 364211 | 54 | 566 |
| 5 | 21 | Survivor | 13 | 1 | 4762 | 14085 | 271 | 210 |
| 6 | 21 | Survivor | 34 | 171 | 660 | 120267 | 47 | 541 |
| No VRP + IbAr10200 | 1 | 4 | Fatal | NS | 1530 | NS | 0 | NS | 76 |
| 2 | 4 | Fatal | 0 | 26 | 0 | 0 | 1 | 41 |
| 3 | 4 | Fatal | 0 | 16 | 3 | 2 | 0 | 19 |
| No VRP + Oman-97 | 1 | 21 | Survivor | 0 | 117 | 11 | 129585 | 15 | 1945 |
| 2 | 21 | Survivor | 0 | 402 | 4 | 133942 | 15 | 2579 |
| 3 | 21 | Survivor | 2 | 161 | 4 | 59920 | 6 | 3111 |
| No VRP + Turkey | 1 | 7 | Fatal | 4 | 3922 | 3 | 3279 | 10 | 4891 |
| 2 | 7 | Fatal | 0 | 2654 | 1 | 7363 | 9 | 5551 |
| 3 | 7 | Fatal | 0 | 3019 | 0 | 7367 | 8 | 4833 |

IgM and IgG antibodies against CCHFV NP and IgG against CCHFV Gc in plasma obtained 24 days after vaccination (Pre), or at disease end-point or completion of study at 21 dpi (Post). Values presented in AAU. All animals were challenged subcutaneously with 1 × 102 TCID50 of indicated CCHFV strains 28 days post VRP vaccination (1 × 105 TCID50) or mock vaccination (No VRP). Pre-challenge blood samples from unvaccinated mice (No VRP) were used to determine cut-off values. N/A, not applicable; NS, no sample.

**Supplementary Methods:**

**Ethics statement**

All animal procedures were approved by the CDC Institutional Animal Care and Use Committee and conducted in accordance with the Guide for the Care and Use of Laboratory Animals at an AAALAC-International accredited facility. Procedures conducted with CCHFV or CCHFV-infected animals were performed in the CDC biosafety level 4 laboratory.

**Viruses**

For CCHFV strain comparison or heterologous challenge experiments, mice were inoculated with Turkey-200406546 (GenBank: KY362517, KY362519, KY362515), UAE-199812347 (GenBank: MF289419, MF289418, MF289417), Oman-199723179, Oman-199809166 (GenBank: KY362516, KY362518, KY362514), or recombinant IbAr10200 based on wild-type IbAr10200 (GenBank KJ648914, KJ648915, and KJ648913)(Bergeron et al., 2015). Passage histories for stocks used in strain comparison experiments: recombinant IbAr10200: rescued in Huh7 cells, passaged 3× in BSR-T7/5 cells; Turkey: 1× suckling mouse brain, 1× SW-13 cells; UAE: 2× Vero-E6, 1× SW-13 cells; Oman-97: 1× Vero-E6, 1× SW-13 cells; and Oman-98: 2× Vero-E6, 1× SW-13 cells. Passage histories for stocks used in heterologous protection experiments: recombinant IbAr10200: as above; Oman-97: 1× suckling mouse brain, 1× BSR-T7/5 cells; and Turkey: 1× suckling mouse brain, 1× BSR-T7/5 cells. Viral titers were calculated as 50 percent tissue culture infective dose (TCID50) (Reed and Muench, 1938), and were determined in parallel in either SW-13 cells by observing cytopathic effects, or in BSR-T7/5 cells by measuring indirect immunofluorescence (Scholte et al., 2017). All recombinant virus work was approved by the CDC Institutional Biosafety Committee. All virus stocks were verified by next-generation sequencing and confirmed mycoplasma free.

**VRP production and titration**

Six-well plates were seeded with 3.5 × 105 Huh7 cells/well 1 day prior to transfection in 3 mL of DMEM supplemented with 1% non-essential amino acids, 1 mM sodium pyruvate, and 10% FBS. 16–24 h later, cells were transfected with pT7-S (1 μg), pT7-L (1 μg), pCAGGS-L (0.33 μg), pCAGGS-NP (0.66 μg), pCAGGS-GPC-Oman (1 μg), and pCAGGS-T7 (1 μg), combined with 12.5 μL of Mirus LT1 transfection reagent (Mirus Bio, Madison, WI, USA) in 250 μL of OPTI-MEM (Life Technologies, Grand Island, NY, USA). Supernatants containing VRPs were harvested 4–5 days post transfection. VRP stocks were titrated by TCID50 on BSR/T7 cells (Reed and Muench, 1938). Positive wells were scored based on the detection of at least one CCHFV NP positive cells detectable by immunofluorescence using a rabbit anti-NP antibody (#04-0011, IBT Bioservices) and Alexa-488 goat anti-rabbit secondary antibody.

**Phylogenetics**

The full-length S genome segment sequences of CCHFV strains from each of the seven clades (Africa 1, Africa 2, Africa 3, Asia 1, Asia 2, Europe 1, and Europe 2) were aligned and a phylogenetic tree was constructed (neighbor joining tree construction, Jukes-Cantor distance measure, and ×1000 bootstrap measurements) using CLC Genomics Workbench version 9.5.2. Radial phylogenetic tree was drawn using FigTree version 1.4.3.