rVSV-BASED COVID VACCINE FOR THE PREVENTION OF BREAKTHROUGH SARS-CoV-2 INFECTIONS

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BACKGROUND

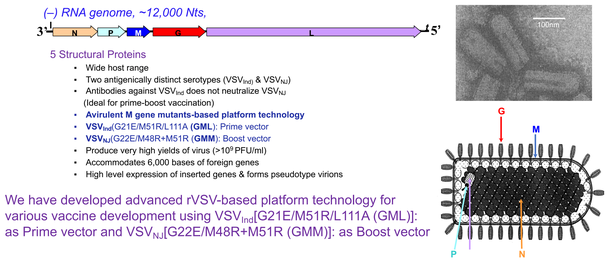
More than 12.7 billion doses of COVID-19 vaccine administrations and over 750 million confirmed cases of SARS-CoV-2 infections as of end of 2022 should have developed adequate levels of herd immunity. However, there have been many new waves of coronavirus infections. The development of safe and effective vaccines to control breakthrough SARS-CoV-2 infections remains an urgent priority worldwide.

METHODS

We have used a recombinant vesicular stomatitis virus (rVSV)-based prime-boost immunization strategy (Fig. 1) to develop an effective COVID-19 vaccine candidate. We have constructed an attenuated recombinant VSV genome carrying the full-length Spike protein (S_F) gene of SARS-CoV-2. Adding the honeybee melittin signal peptide (*msp*) at the N-terminus enhanced the protein expression and adding the VSV G protein transmembrane domain and the cytoplasmic tail (*Gtc*) at the C-terminus of the Spike protein (Fig. 2) allowed efficient incorporation of the Spike protein into pseudotype VSV.

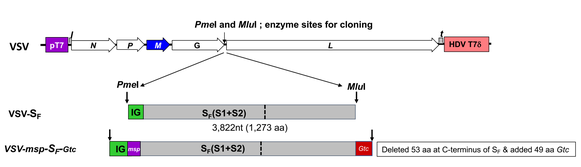
Recombinant rVSV-*msp*-S-*Gtc* induced high levels of potent neutralizing antibodies (nAbs) and CD8⁺ T cell responses, while the full-length Spike with *Gtc* proved to be the superior immunogen. More importantly, rVSV-*msp*-S-*Gtc*-vaccinated animals were completely protected from subsequent SARS-CoV-2 challenges. Furthermore, rVSV-Wuhan and rVSV-Delta vaccines, and an rVSV-Trivalent (mixed rVSV-Wuhan, -Beta and -Delta) vaccine elicited potent nAbs against live SARS-CoV-2 Wuhan (USAWA1), Beta (B.1.351), Delta (B.1.617.2) and Omicron (B.1.1.529) viruses. Heterologous boosting of rVSV-Wuhan with rVSV-Delta induced strong nAb responses against Delta and Omicron viruses, with the rVSV-Trivalent vaccine consistently inducing effective nAbs against all the SARS-CoV-2 variants tested. All rVSV-*msp*-S-*Gtc* vaccines also elicited an immunodominant Spike-specific CD8⁺ T cell response.

Fig. 1 Our Strategy using Vesicular Stomatitis Virus (VSV) System



We have developed advanced rVSV-based platform technology for various vaccine development using VSV$_{Ind}$[G21E/M51R/L111A (GML)]: as Prime vector and VSV$_{NJ}$[G22E/M48R+M51R (GMM)]: as Boost vector

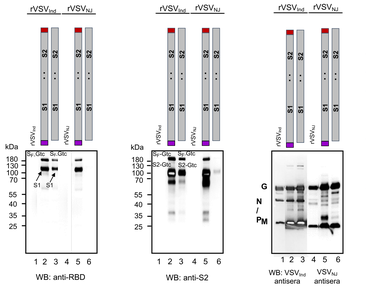
Fig. 2 Construction of rVSV-SARS-CoV-2 Spike



The addition of the honeybee melittin signal peptide (*msp*) at the N-terminus of spike protein S_F allows high levels of expression and efficient secretion. The addition of VSV G protein transmembrane domain and cytoplasmic tail (*Gtc*) at the C-terminus makes S_F protein incorporation into VSV pseudovirions efficiently. These are our unique proprietary approaches for rVSV-based COVID-19 vaccine development.

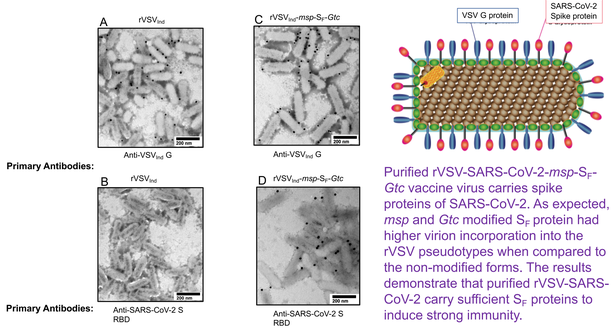
RESULTS

Fig. 3. Incorporation of SARS-CoV-2 Spike Proteins into Purified rVSV Pseudovirion



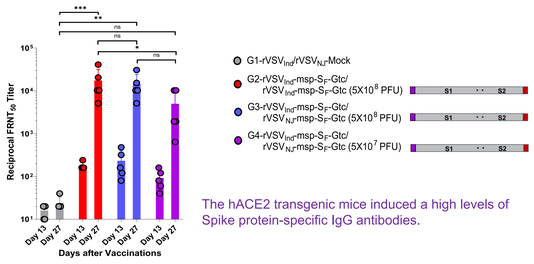
We observed robust expression of full-length Spike protein (S_F), and cleaved S1 and S2 spike proteins in the rVSV$_{Ind}$-SARS-CoV-2 and rVSV$_{NJ}$-SARS-CoV-2 virus infected cells. This indicates that rVSV$_{Ind}$-SARS-CoV-2 and rVSV$_{NJ}$-SARS-CoV-2 COVID vaccines will induce strong immune responses.

Fig. 4 Incorporation SARS-CoV-2 Spike Proteins into rVSV$_{Ind}$-*msp*-S$_F$-*Gtc* Pseudotype



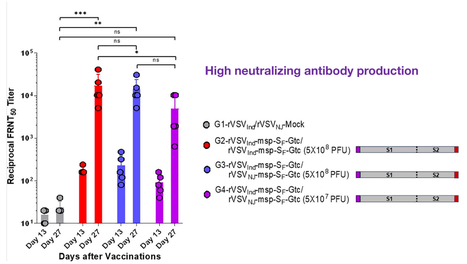
Purified rVSV-SARS-CoV-2-*msp*-S$_F$-*Gtc* vaccine virus carries spike proteins of SARS-CoV-2. As expected, *msp* and *Gtc* modified S_F protein had higher virion incorporation into the rVSV pseudotypes when compared to the non-modified forms. The results demonstrate that purified rVSV-SARS-CoV-2 carry sufficient S_F proteins to induce strong immunity.

Fig. 5 SARS-CoV-2 S Specific IgG induction



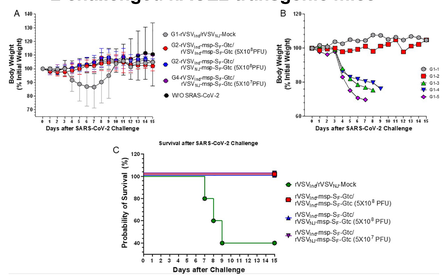
The hACE2 transgenic mice induced a high levels of Spike protein-specific IgG antibodies.

Fig 6 Prime-boost vaccination of hACE2 transgenic mice induces high levels of neutralizing antibodies against SARS-CoV-2



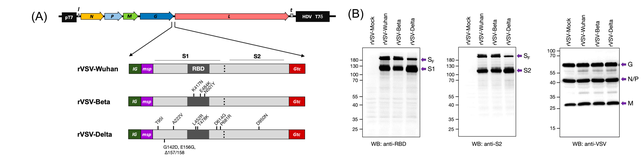
In all immunized groups, neutralizing antibody titers were significantly increased after boost. **Mice immunized with rVSV-*msp*-S$_F$-*Gtc* had the highest average neutralizing antibody titers (1/13,824) after boost compared to all other groups of immunized mice, with neutralizing titers up to a high of 1/40,960.*** These results indicate that the S_F protein induced stronger neutralizing antibody responses, and modification of the S_F with *msp* and *Gtc* enhanced neutralizing antibody production.

Fig. 7 Bodyweight and survival of the vaccinated and SARS-CoV-2 challenged hACE2 transgenic mice



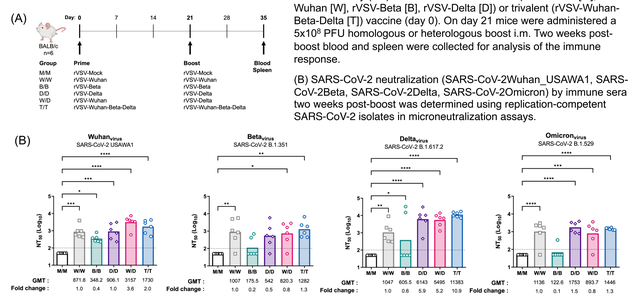
All vaccinated hACE2 transgenic mice survived, and no significant weight loss was observed. This result indicates that rVSV-SARS-CoV-2-*msp*-S$_F$-*Gtc* is a safe vaccine for the boost vaccination.

Fig. 8 Construction & Expression of recombinant rVSV with S_F genes of SARS-CoV-2 Wuhan, Beta, and Delta



(A) Constructs of VSV-SARS-CoV-2 Wuhan (rVSV-Wuhan), Beta (rVSV-Beta), and Delta (rVSV-Delta). Codon-optimized full-length Spike protein gene (S_F) with VSV intergenic sequences (IG), 21 amino acid honeybee melittin signal peptide (*msp*), and 49 amino acid VSV G protein transmembrane domain and cytoplasmic tail (*Gtc*) were inserted into the G and L gene junction of rVSV$_{Ind}$. pT7: Bacteriophage T7 promoter for DNA dependent RNA polymerase. N: VSV Nucleocapsid protein gene. P: VSV Phosphoprotein gene. M: VSV Matrix protein gene. G: VSV Glycoprotein gene. L: VSV Large protein, RNA dependent RNA polymerase gene. I: Leader region in the 3´-end of the VSV genome. t: Trailer region in the 5´-end of the VSV genome. HDV: Hepatitis delta virus ribozyme encoding sequences. Tτδ: Bacteriophage T7 transcriptional terminator sequences. nt: nucleotides. aa: amino acids.
(B) Expression of SARS-CoV-2 Spike proteins in BHK-21 cells at 6 hr post-infection with rVSV-Mock (rVSV), rVSV-Wuhan, rVSV-Beta, and rVSV-Delta.

Fig 9 Immunization with monovalent and trivalent vaccines elicit a broad neutralizing antibody response



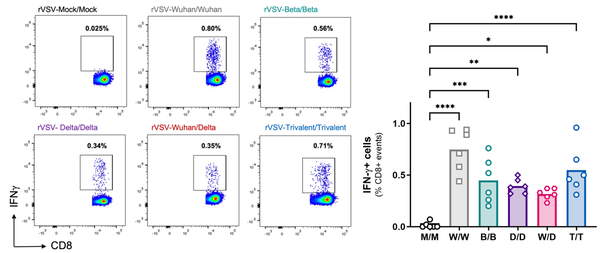
(A) Female BALB/c mice were prime immunized with 5x10^8 PFU intramuscularly (i.m) of either rVSV-monovalent (rVSV-Mock [M], rVSV-Wuhan [W], rVSV-Beta [B], rVSV-Delta [D]) or trivalent (rVSV-Wuhan-Beta-Delta [T]) vaccine (day 0). On day 21 mice were administered a 5x10^8 PFU homologous or heterologous boost i.m. Two weeks post-boost blood and spleen were collected for analysis of the immune response.

(B) SARS-CoV-2 neutralization (SARS-CoV-2Wuhan_USAWA1, SARS-CoV-2Beta, SARS-CoV-2Delta, SARS-CoV-2Omicron) by immune sera two weeks post-boost was determined using replication-competent SARS-CoV-2 isolates in microneutralization assays.

Graphed data are presented as arithmetic mean of log-transformed 50% neutralization titer (NT50). The horizontal dotted lines indicate the limit of detection. Each symbol denotes an individual animal; n=6; 2 independent experiments. Statistical significance was determined by one-way ANOVA with Dunnett's correction for multiple comparisons (*, p < 0.05; **, p<0.01; ***, p< 0.001; **** p<0.0001). Geometric mean NT$_{50}$ (GMT) and the fold increases in GMT NT$_{50}$ versus W/W (rVSV-Wuhan/Wuhan) are shown for each vaccine group.

Prime-boost with the original rVSV-Wuhan monovalent vaccine (rVSV-Wuhan/Wuhan) induced an effective nAb response against matched SARS-CoV-2Wuhan_USAWA1. The remaining rVSV prime-boost combinations, rVSV-Beta/Beta, rVSV-Delta/Delta, rVSV-Wuhan/Delta and rVSV-Trivalent/Trivalent vaccines, also exhibited significant neutralization of SARS-CoV-2Wuhan_USAWA1. The rVSV-Wuhan/Delta heterologous Delta boost regimen exhibited superior neutralization with a 3.6-fold increase compared to rVSV-Wuhan/Wuhan ($p < 0.05$). The rVSV-Trivalent/Trivalent vaccine had mean neutralization 2-fold higher than rVSV-Wuhan/Wuhan, but this difference was not statistically significant. Serum antibodies elicited from rVSV-Beta/Beta vaccination were inefficient at neutralizing matched SARS-CoV-2Beta. Interestingly, at least 5 of 6 mice from the other vaccine regimens were able to cross-neutralize SARS-CoV-2Beta, with 100% of rVSV-Trivalent/Trivalent immunized mice generating a neutralizing antibody response. SARS-CoV-2Delta was neutralized effectively by all vaccine regimens except rVSV-Beta/Beta vaccination. Serum from rVSV-Trivalent/Trivalent immunization exhibited a 10.9-fold increase in neutralization of SARS-CoV-2Delta ($p < 0.05$). Serum from rVSV-Delta/Delta and rVSV-Wuhan/Delta immunizations exhibited 5.9-fold and 5.2-fold higher mean neutralization compared to rVSV-Wuahn/Wuhan, respectively, but these differences were not statistically significant . The most highly mutated variant, SARS-CoV-2Omicron, was neutralized by nAbs induced through prime-boost of all vaccine regimens except rVSV-Beta/Beta. Overall, serum from rVSV-Trivalent/Trivalent immunization was effective at generating nAb responses in 100% of mice for all SARS-CoV-2 viruses tested (SARS-CoV-2Wuhan_USAWA1, SARS-CoV-2Beta, SARS-CoV-2Delta and SARS-CoV-2Omicron) (Fig. 9).

Fig. 10 Monovalent and Trivalent vaccines generate a cognate CD8⁺ T cell response



Female BALB/c mice were prime immunized with 5x10^8 PFU intramuscularly (i.m) of either rVSV-monovalent (rVSV-Mock, rVSV-Wuhan, rVSV-Beta, rVSV-Delta) or trivalent (rVSV-Wuhan-Beta-Delta) vaccine (day 0). On day 21 mice were administered a 5x10^8 PFU homologous or heterologous boost i.m. Two weeks post-boost splenocytes were prepared and stimulated with SARS-CoV-2 specific epitope (S535 – KNKCVNFNF) *ex vivo*. IFN-γ production was determined following Brefeldin A treatment via intracellular cell staining and flow cytometry

All rVSV prime-boost vaccine regimens elicited a significant cognate CD8+ T cell response to the MHCI-restricted peptide S_{535} when compared to rVSV-Mock/Mock immunization CD8⁺ T cell responses were higher in rVSV-Wuhan/Wuhan immunized mice compared to rVSV-Beta/Beta, rVSV-Delta/Delta, nd rVSV-Wuhan/Delta immunized mice; but equivalent to rVSV-Trivalent/Trivalent immunized mice IFN-γ production was not detected 14 days post-boost for two additional MHCI-restricted peptides and two MHCII-restricted peptides.
Overall, this indicates that all vaccinated mice mounted a SARS-CoV-2 spike-specific CD8⁺ T cell response.

CONCLUSIONS

Recombinant VSV with chimeric rVSV-*msp*-S$_F$-*Gtc* induced high levels of potent neutralizing antibodies (nAbs) and CD8⁺ T cell responses in immunized mice, while the full-length Spike with *Gtc* proved to be the superior immunogen. Furthermore, rVSV-*msp*-S-*Gtc*-vaccinated animals were completely protected from subsequent SARS-CoV-2 challenges. rVSV-Wuhan and rVSV-Delta vaccines, and an rVSV-Trivalent (mixed rVSV-Wuhan, -Beta and -Delta) vaccine elicited potent nAbs against live SARS-CoV-2 Wuhan (USAWA1), Beta (B.1.351), Delta (B.1.617.2) and Omicron (B.1.1.529) viruses. All rVSV-*msp*-S$_F$-*Gtc* vaccines also elicited an immunodominant Spike-specific CD8⁺ T cell response.

Recombinant VSV vaccines targeting SARS-CoV-2 variants of concern can be considered as an effective booster vaccine in the global fight against COVID-19.

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