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Letter

A single dose of recombinant VSV-RABV$_G$ vaccine provides full protection against RABV challenge

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Dear Editor,

Rabies virus (RABV) is an enveloped, non-segmented, and single-stranded RNA virus that belongs to the genus *Lyssavirus* within the *Rhabdoviridae* family (Douglas et al., 2013). RABV causes rabies, and although vaccines are available, rabies continues to be a global public health concern causing more than 60,000 human deaths each year (WHO, 2013). There is a high prevalence especially in developing countries in Asia and Africa (Hampson et al., 2015; Singh et al., 2017). In most cases, RABV is transmitted through the direct contact of broken skin or the mucous membrane with the saliva of infected dogs. Unless the wound is promptly cleaned and post-exposure prophylaxis is administered, the human victim may develop encephalitis that is nearly always fatal.

Licensed rabies vaccines for human use are based on inactivated purified RABV, which is grown either in tissue culture or in embryonic duck or chicken eggs. Rabies vaccines can be given intramuscularly (i.m.) or at a 5–10-fold lower dose intradermally. For pre-exposure prophylaxis, the vaccine is given typically three times: on days 0, 7, and 21 or 28. Efforts are underway to change the plan to a two-dose regimen for which individuals are vaccinated into two sites on days 0 and 7. Moreover, previously vaccinated individuals frequently require a booster as a post exposure prophylaxis measure (Ertl, 2019).

The recombinant vesicular stomatitis virus (rVSV), which was developed by John Rose and Michael Whitt, is widely used as a vaccine platform for several viral pathogens (Lawson et al., 1995). The United States Food and Drug Administration and the European Medicines Agency approved rVSV-based ebolavirus vaccine in 2019, which is considered a hallmark of rVSV-based vaccines. Recently, we have reported that one dose of an rVSV vaccine is sufficient to induce robust protective immunity against coxsackievirus B3 infection. The efficacy of rVSV-based vaccines was also demonstrated by others in rVSV-based SARS-CoV-2 vaccine (Wu et al., 2014; Yahalom-Ronen et al., 2020). In this study, an rVSV was generated in which the VSV G envelope protein was replaced by the RABV G protein, and its ability to induce the humoral response and protection were then investigated in mice (Supplementary Materials and Methods).

To generate rVSV expressing the RABV G protein, an *Mlu*I and *Xho*I flanked DNA fragment, including the RABV *G* gene (GenBank: M13215.1) was synthesized by GENEWIZ. The original VSV G was excised by *Mlu*I and *Xho*I from an attenuated backbone pXN2^{M51R}-GFP carrying M51R mutation in *M* gene of VSV genome (Wu et al., 2019). The synthetic DNA fragment was then ligated into the digested pXN2^{M51R}-GFP plasmid, generating the pXN2-RABV$_G$ plasmid. Recovery of a replication-competent VSV-RABV$_G$ (Fig. 1A) with the replacing of RABV G protein was conducted as described previously using co-transfection pP, pN, pL plasmids together with pXN2-RABV$_G$ in BHK cells (Lawson et al., 1995). Vero cells were then infected with VSV-RABV$_G$ or control VSV-GFP at multiplicities of infection of 10. Two days post infection, the cytopathic effect was observed and many of the VSV- RABV$_G$ infected cells became round and broken was obvious (Fig. 1B). Western blotting was further performed using an anti-RABV G primary antibody to confirm the expression of RABV G protein in infected cells (Supplementary Fig. S1).

To investigate the toxicity of VSV-RABV$_G$ in immunized mice, the body weights of Balb/c mice were immunized intranasally with 1×10^5, 1×10^6, and 1×10^7 plaque-forming units (PFUs) of the virus in 50 μL of phosphate-buffered saline. The body weights of the mice immunized with 1×10^6 and 10^7 PFUs of the virus both decreased early after immunization. However, the body weights increased after 2 days in mice immunized with 1×10^6 PFU whereas there was a large decrease in mice immunized with 10^7 PFU after 8 days (Fig. 1C). This suggested that 1×10^6 PFU would be an appropriate dose for immunizing mice against RABV without obvious toxicity. The expression of the RABV G protein

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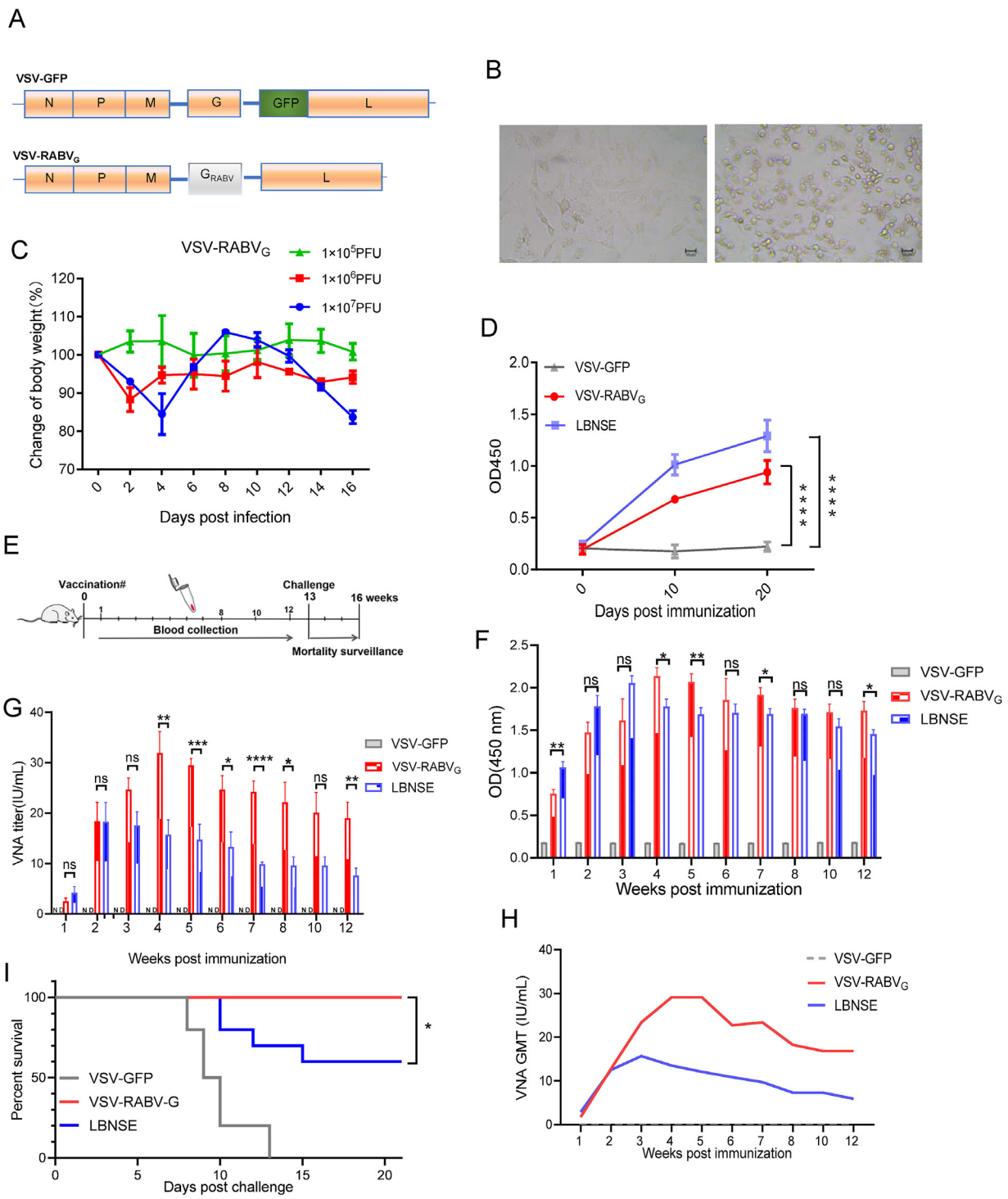

Fig. 1. Humoral immunity and protection induced by a single dose of VSV-RABV$_G$. **A** Schematic of VSV-RABV$_G$ and VSV-GFP. **B** Vero cells were infected with VSV-RABV$_G$ at multiplicities of infection of 10. Two days post-infection, the cells were observed under microscopy Scale bar: 50 μm. **C** Body weight changes of VSV-RABV$_G$-infected mice. Mice were infected intranasally with 1×10^5, 1×10^6 and 1×10^7 PFUs of the VSV-RABV$_G$ recombinant virus. The body weights of infected mice were measured daily for 16 days. **D** Short-term humoral response in immunized mice. Mice were intranasally immunized with 1×10^6 PFUs of VSV-RABV$_G$ or VSV-GFP, or immunized intramuscularly with 1×10^6 PFUs of LBNSE. Serum was collected at the indicated times for detection of the RABV G antibody level by an enzyme-linked immunosorbent assay. Each group contained five mice. **E** Schematic of the long-term detection of the VSV-RABV$_G$-induced humoral response. The serum of immunized mice was collected at the indicated time points for 12 weeks post-immunization. At 13 weeks post-immunization, the mice were challenged intracerebrally with 50 × LD_{50} of DRV-Mexico in at a volume of 30 μL. **F**–**H** Long-term humoral response in immunized mice. Mice were immunized as in "**E**". The serum of immunized mice was collected weekly for 12 weeks, then assayed for the (**F**) total RABV G antibody level, (**G**) virus neutralizing antibody (VNA) level, and (**H**) VNA geometric mean titer (GMT). Each group contained 10 mice. **I** Immunized mice were challenged intracerebrally with 50 × LD_{50} of DRV-Mexico in a volume of 30 μL 13 weeks post-immunization and the survival ratio was monitored daily for 3 weeks. Each group contained 10 mice. Survival curves were analyzed using the log-rank (Mantel-Cox) test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, *****$P < 0.0001$, ns: no significance.

was examined in immunized mice 24 days after the immunization with 1×10^6 PFUs of the VSV-RABV$_G$. High expression levels of the RABV G protein were observed in the kidney, lung, spleen, and heart, while low levels were detected in the stomach, small intestine. As RABV is neurotoxic, it was worth noting that the RABV G protein expression was rare in the brain (Supplementary Fig. S2). The symptoms of immunized mice were normal and they did not get the nervous, hair shedding, suggesting the safety of VSV-RABV$_G$ immunization under 1×10^6 PFUs dosage in mice.

Next, to investigate the immune response elicited by the VSV-RABV$_G$, Balb/c mice were immunized intranasally with a single dose of 1×10^6 PFUs of VSV or an intramuscular injection of 1×10^6 PFUs of LBNSE, which is a live-attenuated RABV vaccine (Li et al., 2020). The serum of the immunized mice was collected and assayed for RABV G-specific antibody production by an enzyme-linked immunosorbent assay (Fig. 1D). The RABV G-specific antibody was detected in the LBNSE and VSV-RABV$_G$ immunized mice at 10 and 20 days post-immunization. The amount of induced specific antibody increased over time. And there was no RABV G-specific antibody detected in VSV-GFP immunized mice. The antibody level in each mouse at different time points is plotted in Supplementary Fig. S3. Because inactivated vaccines fail to induce a strong immune response and do not provide long-term protection against RABV infection, vaccine recipients are required three to four injections over time to achieve ideal immune protection. There is a report showing that a Newcastle disease virus-vectored rabies vaccine provides a long-lasting protection in dogs and cats (Ge et al., 2011). Therefore, we extended the detection time to 12 weeks after immunization (Fig. 1E). We found that the peak RABV G-specific antibody level in the VSV-RABV$_G$ group was higher than that in the LBNSE group, but the induction was slightly slower (4 weeks to peak vs. 3 weeks to peak, respectively, Fig. 1F). It is generally assumed that the immunization with the inactivated RABV elicits a species-independent type-2 response with high levels of type-2-associated IgG1 RABV-specific antibodies (Bommier et al., 2020), while the live attenuated RABV vaccines induce type-1 responses (Lebrun et al., 2017). Measuring the IgG subtype elicited by VSV-RABV$_G$ immunization demonstrated that IgG2b was the most prevalent antigen-specific subtype, followed by IgG1 and IgG2a (Supplementary Fig. S4).

Virus neutralizing antibodies (VNAs) were measured as previously described (Zhang et al., 2019). It was apparent that VSV-RABV$_G$ induced higher VNA levels than LBNSE after 4 weeks immunization. This is critical because VNAs are believed to be one of the key factors to evaluate rabies vaccine efficacy. Even at 12 weeks post-immunization, the VNA titer in the VSV-RABV$_G$ group remained greater than 20 IU/mL, which is much higher than 0.5 IU/mL, the suggested least titer needed for immune protection (Fig. 1G). Similar results were obtained when VNA geometric mean titers were calculated (Fig. 1H).

To investigate whether higher VNA titers correlated with better protection, mice were injected intracerebrally with $50 \times LD_{50}$ of DRV-Mexico (a pathogenic RABV strain) in a volume of 30 μL 13 weeks post-immunization. The mice were then monitored for survival over the following 3 weeks. All mice ($n = 10$) died in the control VSV-GFP immunized group, while 60% of the mice survived in the LBNSE immunized group. Surprisingly, 100% of mice that were immunized with VSV-RABV$_G$ survived, demonstrating its potent efficacy against RABV infection (Fig. 1I).

To induce a potent immune response that protects against RABV infection, according to the guidelines for rabies prevention and technical control (China CDC, 2016), the currently used inactivated RABV vaccine must be given three times for pre-exposure prophylaxis or five times for post-exposure-prophylaxis. A very recent study developed a replication-deficient VSV based RABV vaccine, with which two doses of immunization were required to induce the humoral immunity and the protection in challenged mice was still unknown (Park and Shin, 2021). Thus, the VSV-based replication-competent RABV vaccine we developed in the current study is very meaningful because, in contrast to inactivated vaccines, only one immunization dose elicited strong and long-lasting VNA production, and can protect all the DRV-Mexico -infected mice. An additional advantage is that VSV-RABV$_G$ can be administered intranasally; thus, avoiding the use of a needle. This is particularly valuable for people who live in rural regions with limited access to health care. Nevertheless, we still do not know the therapeutic effects of the vaccine when the infection occurs prior to vaccination because RABV-infected mice die as early as 6 days that there may be not enough protective immunity response generated by the vaccine. Additionally, although rVSV based vaccine has been successfully applied for the prevention of ebolavirus infection, considering both VSV and RABV possess neurotropism, the safety of VSV-RABV$_G$ needs to be further investigated in non-human primates. In summary, we provide here an efficacious and convenient VSV-based vaccine with the potential for rabies control.

Footnotes

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References

Bommier, E., Chapat, L., Guiot, A.L., Hilaire, F., Cariou, C., Poulet, H., Pialot, D., De Luca, K., 2020. Multivariate analysis of the immune response to different rabies vaccines. Vet. Immunol. Immunopathol. 220, 109986.

China CDC, 2016. Technical guidelines for human rabies prevention and contro. https://www.chinacdc.cn/zxdt/201602/t20160201_125012.html (accessed on 20 June 2021).

Douglas S, Lyles IVK, Rupprecht CE. 2013. Rhabdoviridae, p 885–922. In Knipe DM, Howley PM, Cohen JL, Griffin DE, Lamb RA, Martin MA, Racaniello VR, Roizman B (ed), Fields Virology, 6th ed, vol 1. Lippincott Williams and Wilkens, Philadelphia, PA

Ertl, H.C.J., 2019. New rabies vaccines for use in humans. Vaccines (Basel) 7 (2), 54.

Ge, J., Wang, X., Tao, L., Wen, Z., Feng, N., Yang, S., Xia, X., Yang, C., Chen, H., Bu, Z., 2011. Newcastle disease virus-vectored rabies vaccine is safe, highly immunogenic, and provides long-lasting protection in dogs and cats. J. Virol. 85, 8241–8252.

Hampson, K., Coudeville, L., Lembo, T., Sambo, M., Kieffer, A., Attlan, M., Barrat, J., Blanton, J.D., Briggs, D.J., Cleaveland, S., Costa, P., Freuling, C.M., Hiby, E., Knopf, L., Leanes, F., Meslin, F.X., Metlin, A., Miranda, M.E., Muller, T., Nel, L.H., Recuenco, S., Rupprecht, C.E., Schumacher, C., Taylor, L., Vigilato, M.A., Zinsstag, J., Dushoff, J., Global Alliance for Rabies Control Partners for Rabies, P, 2015. Estimating the global burden of endemic canine rabies. PLoS Neglected Trop. Dis. 9, e0003709.

Lawson, N.D., Stillman, E.A., Whitt, M.A., Rose, J.K., 1995. Recombinant vesicular stomatitis viruses from DNA. Proc. Natl. Acad. Sci. U. S. A. 92, 4477–4481.

Lebrun, A., Garcia, S., Li, J., Kean, R.B., Hooper, D.C., 2017. Protection against CNS-targeted rabies virus infection is dependent upon type-1 immune mechanisms induced by live-attenuated rabies vaccines. Tropical Med. Infect. Dis. 2, 22.

Li, Y., Zhao, L., Sui, B., Luo, Z., Zhang, Y., Wang, Y., 2020. Recombinant rabies virus overexpressing OX40-ligand enhances humoral immune responses by increasing T follicular helper cells and germinal center B cells. Vaccines 8, 144.

Park, J.E., Shin, H.J., 2021. Immunogenicity of replication-deficient vesicular stomatitis virus based rabies vaccine in mice. Vet. Q. 41, 202–209.

Singh, R., Singh, K.P., Cherian, S., Saminathan, M., Kapoor, S., Manjunatha Reddy, G.B., Panda, S., Dhama, K., 2017. Rabies - epidemiology, pathogenesis, public health concerns and advances in diagnosis and control: a comprehensive review. Vet. Q. 37, 212–251.

WHO, 2013. WHO Expert Consultation on Rabies. Second report. World Health Organ Tech Rep Ser, pp. 1–139. back cover.

Wu, C., Wu, M., Liang, M., Xiong, S., Dong, C., 2019. A novel oncolytic virus engineered with PD-L1 scFv effectively inhibits tumor growth in a mouse model. Cell. Mol. Immunol. 16, 780–782.

Wu, F., Fan, X., Yue, Y., Xiong, S., Dong, C., 2014. A vesicular stomatitis virus-based mucosal vaccine promotes dendritic cell maturation and elicits preferable immune

response against coxsackievirus B3 induced viral myocarditis. Vaccine 32, 3917–3926.
Yahalom-Ronen, Y., Tamir, H., Melamed, S., Politi, B., Shifman, O., Achdout, H., Vitner, E.B., Israeli, O., Milrot, E., Stein, D., Cohen-Gihon, I., Lazar, S., Gutman, H., Glinert, I., Cherry, L., Vagima, Y., Lazar, S., Weiss, S., Ben-Shmuel, A., Avraham, R., Puni, R., Lupu, E., Bar-David, E., Sittner, A., Erez, N., Zichel, R., Mamroud, E., Mazor, O., Levy, H., Laskar, O., Yitzhaki, S., Shapira, S.C., Zvi, A., Beth-Din, A., Paran, N., Israely, T., 2020. A single dose of recombinant VSV-G-spike vaccine provides protection against SARS-CoV-2 challenge. Nat. Commun. 11, 6402.
Zhang, Y., Yang, J., Li, M., Cui, M., Fu, Z.F., Zhao, L., Zhou, M., 2019. A recombinant rabies virus expressing fms-like tyrosine kinase 3 ligand (Flt3L) induces enhanced immunogenicity in mice. Virol. Sin. 34, 662–672.