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Parainfluenza Virus 5 Expressing the G Protein of Rabies Virus Protects Mice after Rabies Virus Infection

Ying Huang,[a] Zhenhai Chen,[b] Junhua Huang,[a] ZhenFang Fu,[a] Biao He[b]

Department of Pathology[a] and Department of Infectious Diseases,[b] University of Georgia College of Veterinary Medicine, Athens, Georgia, USA

Rabies remains a major public health threat around the world. Once symptoms appear, there is no effective treatment to prevent death. In this work, we tested a recombinant parainfluenza virus 5 (PIV5) strain expressing the glycoprotein (G) of rabies (PIV5-G) as a therapy for rabies virus infection: we have found that PIV5-G protected mice as late as 6 days after rabies virus infection. PIV5-G is a promising vaccine for prevention and treatment of rabies virus infection.

Rabies is caused by rabies virus (RABV) infection and is almost always fatal once symptoms occur. Despite significant scientific progress, more than 55,000 human fatalities are reported annually, and millions of others require postexposure treatment (1, 2). Postexposure prophylaxis (PEP) with vaccines and antirabies immunoglobulin is very efficacious when it is initiated within a few days (but as soon as possible) (3). However, delayed treatment with the rabies vaccines currently in use may actually accelerate the development of rabies (4). It is widely accepted that there is no effective treatment, and rabies is almost invariably fatal once clinical symptoms of rabies develop (4). Thus, new modalities are needed to prevent and treat clinical rabies. Recently, laboratory-attenuated RABV (5) and recombinant RABV expressing three copies of glycoprotein (G) (6) or granulocyte-macrophage colony-stimulating factor (GM-CSF) (7, 8) have been directly injected into the brains of mice and were found to stimulate virus neutralization antibody (VNA) production and enhance blood-brain barrier (BBB) permeability, resulting in the clearance of RABV from the central nervous system (CNS) and prevention of the development of rabies after infection with RABV. These studies indicate that it might be possible to develop therapeutics for treatment of clinical rabies. How-

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Address correspondence to ZhenFang Fu, zhenfu@uga.edu, or Biao He, bhe@uga.edu.

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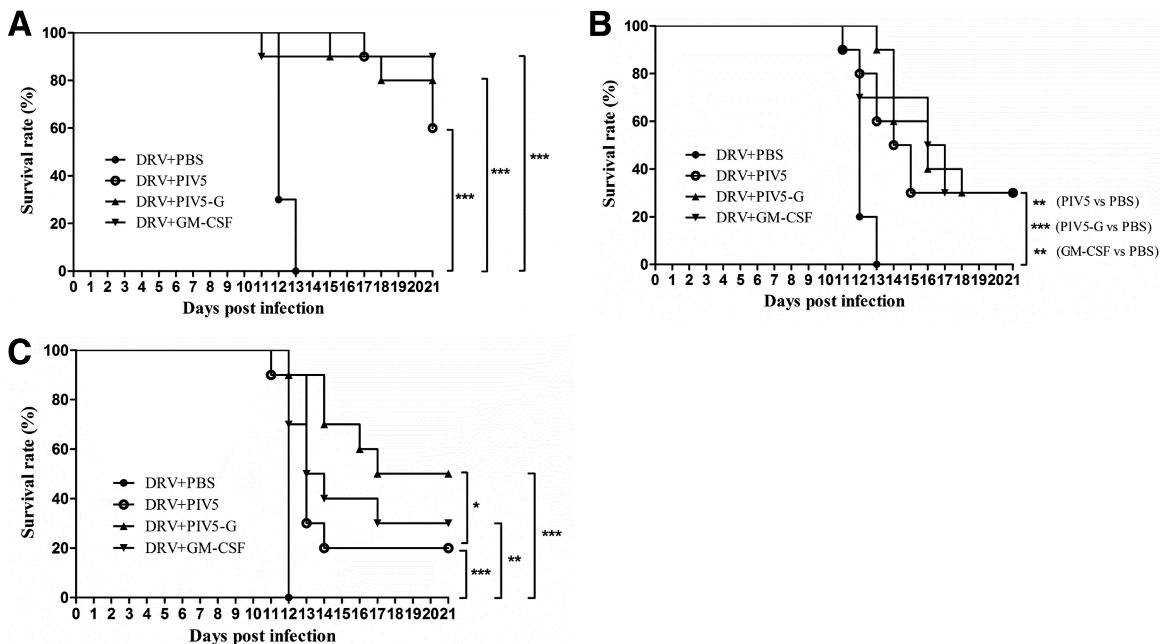

FIG 1 Protective efficacy of PIV5-G administered after i.m. infection with DRV. Mice (in a group of 10) at 4 to 6 weeks of age were infected i.m. with 50 $IMLD_{50}$ DRV and treated with PBS and 10^7 PFU of PIV5 or PIV5-G or 10^7 FFU of LBNSE–GM-CSF by the i.c. route at different time points postinfection (4 dpi [A], 5 dpi [B], and 6 dpi [C]). Infected and treated mice were observed daily for 21 days, and survivorship was recorded and analyzed. Asterisks indicate significant differences between the indicated experimental groups as calculated by the log-rank test as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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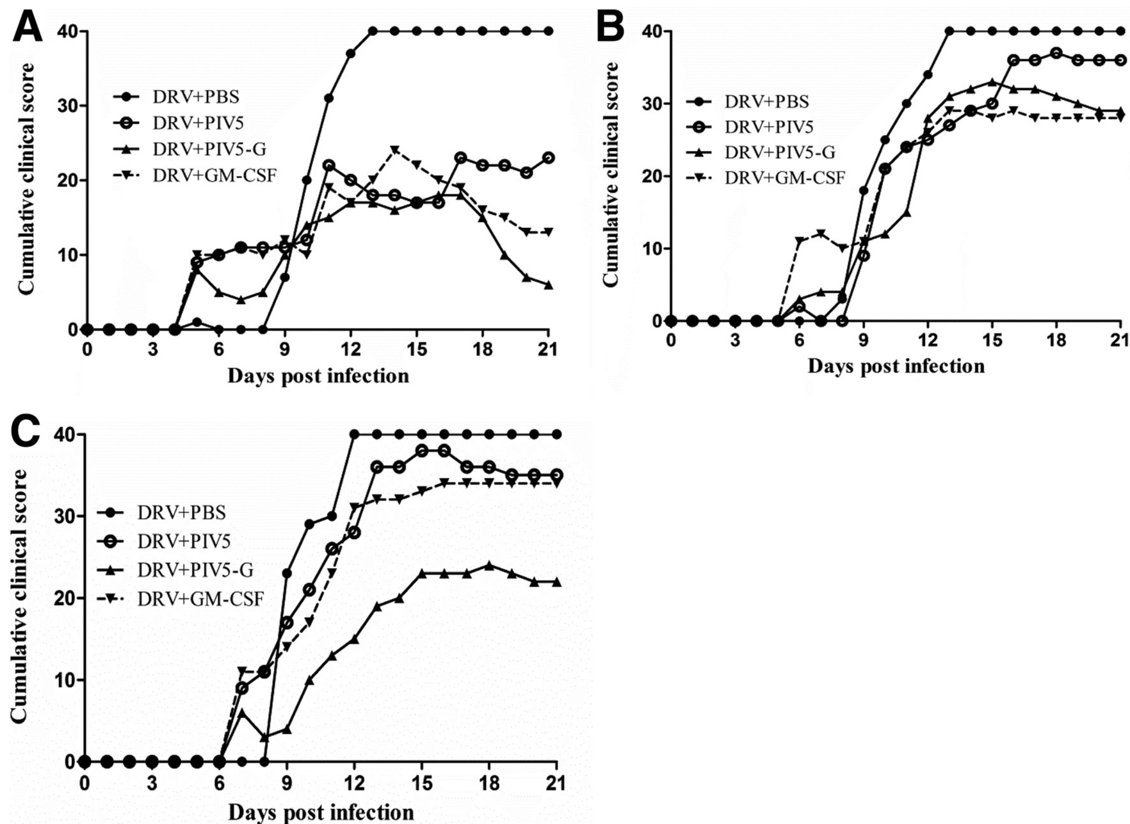

FIG 2 Cumulative clinical symptoms in mice treated with PIV5-G after i.m. infection with DRV. Mice (group of 10) at 4 to 6 weeks of age were infected i.m. with 50 $IMLD_{50}$ DRV and treated with PBS and 10^7 PFU of PIV5 or PIV5-G or 10^7 FFU LBNSE–GM-CSF by the i.c. route at different time points postinfection (4 dpi [A], 5 dpi [B], and 6 dpi [C]). Infected and treated mice were observed daily for 21 days, and the cumulative clinical scores were recorded. 0 = no sign of disease, 1 = ruffled hair, 2 = motor impairment (hogback, unstable gait, and lack of coordination of the hind legs), 3 = one paralyzed hind leg, 4 = two paralyzed hind legs and death.

ever, it is highly unlikely that a live RABV recombinant vaccine will ever be approved for injection into a human brain due to safety concerns.

Parainfluenza virus 5 (PIV5) is a member of the *Rubulavirus* genus of the family *Paramyxoviridae*, which includes mumps virus (MuV) and human parainfluenza virus type 2 (HPIV2) and HPIV4 (9). PIV5 is a good vector candidate for vaccine development. It is believed that PIV5 may contribute to kennel cough in dogs (10–14). Even though infection of dogs with PIV5 did not lead to kennel cough (15, 16), kennel cough vaccines containing live PIV5 have been used in dogs for over 40 years without safety concern for animals or humans. PIV5 infects a large number of mammals without being associated with any diseases, except kennel cough in dogs (10–14). Humans have been exposed to PIV5, likely due to wide use of kennel cough vaccines, which contain live PIV5, and dogs can shed virus after vaccination (15). Because PIV5 does not have a DNA phase in its life cycle, its use avoids the possible unintended consequences of genetic modifications of host cell DNA through recombination or insertion. In comparison to positive-strand RNA virus genome structures, the genome structure of PIV5 is stable. A recombinant PIV5 expressing green fluorescent protein (GFP) has been generated, and the GFP gene was maintained for more than 10 generations (the duration of the experiment) (17). PIV5 can be grown to 8×10^8 PFU/ml, indicating its potential as a cost-effective and safe vaccine vector that may be used in mass production. We have found that PIV5-based influenza virus and respiratory syncytial virus vaccines are efficacious (18, 24, 25). Finally, we have found that recombinant PIV5 expressing G of rabies virus provided complete protection in mice against lethal rabies challenge (19).

To examine whether PIV5-G can be effective to treat mice after RABV infection, we infected mice by the intramuscular (i.m.) route with a wild-type virus (DRV strain) at a dose of 50 $IMLD_{50}$s (median lethal doses administered via intramuscular [i.m.] infection) at the right hind limb. In this infection model, rabies virus reaches brain of the infected mice within 3 days postinfection (dpi) (20). At 4, 5, and 6 days after infection, mice were injected intracerebrally (i.c.) with phosphate-buffered saline (PBS) and 10^7 focus-forming units (FFU) of PIV5, PIV5-G, or LBNSE–GM-CSF (an attenuated RABV expressing GM-CSF [21]) by the i.c. route at 4, 5, and 6 dpi. Mice were monitored daily for 20 days for developing disease and death. By dpi 6 to 8, animals began to develop clinical signs, such as ruffled fur, trembling and shaking, uncoordinated movement, and paralysis. Mice were humanely sacrificed when they developed complete paralysis by dpi 10 to 13. As shown in Fig. 1, 100% of the mice infected with DRV by the i.m. route, but treated with medium by the i.c. route at 4 dpi, developed rabies and succumbed to infection by 13 dpi. Only 10% of DRV-infected mice developed any clinical signs when treated i.c. with LBNSE–GM-CSF at 4 dpi. Significantly more (80%) mice survived the infection when treated with PIV5-G at 4 dpi. When the treatment was initiated at 5 dpi, 30% of the mice treated with

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PIV5-G survived and 30% of the LBNSE–GM-CSF-treated mice survived. When the treatment was initiated at 6 dpi, 50% of the mice treated with PIV5-G survived and 30% of LBNSE–GM-CSF-treated mice were protected. Consistent with the survival results, PIV5-G reduced clinical symptoms of rabies infection (Fig. 2). These results demonstrate that PIV5-G was as effective as LBNSE–GM-CSF in treating rabies virus-infected mice.

To clear rabies virus from the CNS, two factors are absolutely needed, i.e., enhancement of BBB permeability and the production of virus-neutralizing antibodies (8). Our previous studies showed that recombinant RABVs expressing chemokines/cytokines, e.g., GM-CSF (LBNSE–GM-CSF), activate/recruit dendritic cells (DCs) and enhance protective immune responses when given before and after challenge (21, 22). In this work, we used only PIV5 expressing G. We can further improve the efficacy of PIV5-G by expression of GM-CSF as well.

In addition to the fact that PIV5 is not known to cause diseases in humans, one additional advantage of using PIV5-based rabies vaccine as a therapy is that PIV5-G can be combined with anti-rabies antibody. Anti-rabies antibody can and does limit the effectiveness of live RABV (21, 22), and anti-rabies antibody is unlikely to prevent PIV5-G replication because PIV5-G replication does not require the G protein of RABV. Furthermore, the presence of anti-PIV5 antibody did not affect the effectiveness of PIV5-based vaccine (23). Thus, we can further improve the efficacy of PIV5-G by combining PIV5-G treatment with the use of anti-rabies antibodies. The rationale for this combined therapy is that an individual vaccinated with PIV5-G may not be able to generate anti-rabies antibodies fast enough and that, by adding exogenous anti-rabies antibodies at the time of immunization, it will be possible to bridge the gap in time before PIV5-G-induced anti-rabies antibodies are produced.

In summary, in this work, we have demonstrated that PIV5-G is as effective as LBNSE–GM-CSF, the most efficacious treatment reported in literature for animals after rabies infection, in treating mice after rabies infection. Furthermore, the efficacy of PIV5-G treatment can be further improved by changing the G insertion site within the PIV5 genome, modifying the PIV5 genome, coexpressing GM-CSF, and combining such treatment with anti-rabies antibody treatment.

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REFERENCES

1. **Meslin FX, Fisbein DB, Matter HC.** 1994. Rationale and prospects for rabies elimination in developing countries. Curr Top Microbiol Immunol **187:**1–26.
2. **Anonymous.** 1993. World survey of rabies 27. Veterinary Public Health Unit, WHO, Geneva, Switzerland.
3. **WHO.** 2010. Fact sheet no. 99. WHO, Geneva, Switzerland.
4. **Rupprecht CE, Hanlon CA, Hemachudha T.** 2002. Rabies re-examined. Lancet Infect Dis **ii:**327–343. http://dx.doi.org/10.1016/S1473-3099(02)00287-6.
5. **Phares TW, Kean RB, Mikheeva T, Hooper DC.** 2006. Regional differences in blood-brain barrier permeability changes and inflammation in the apathogenic clearance of virus from the central nervous system. J Immunol **176:**7666–7675. http://dx.doi.org/10.4049/jimmunol.176.12.7666.
6. **Faber M, Li J, Kean RB, Hooper DC, Alugupalli KR, Dietzschold B.** 2009. Effective preexposure and postexposure prophylaxis of rabies with a highly attenuated recombinant rabies virus. Proc Natl Acad Sci U S A **106:**11300–11305. http://dx.doi.org/10.1073/pnas.0905640106.
7. **Wen Y, Wang H, Wu H, Yang F, Tripp RA, Hogan RJ, Fu ZF.** 2011. Rabies virus expressing dendritic cell-activating molecules enhances the innate and adaptive immune response to vaccination. J Virol **85:**1634–1644. http://dx.doi.org/10.1128/JVI.01552-10.
8. **Wang H, Zhang G, Wen Y, Yang S, Xia X, Fu ZF.** 2011. Intracerebral administration of recombinant rabies virus expressing GM-CSF prevents the development of rabies after infection with street virus. PLoS One **6:**e25414. http://dx.doi.org/10.1371/journal.pone.0025414.
9. **Lamb RA, Kolakofsky D.** 2001. *Paramyxoviridae*: the viruses and their replication. *In* Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE (ed), Fields virology, 4th ed. Lippincott Williams & Wilkins, Philadelphia, PA.
10. **Binn LN, Eddy GA, Lazar EC, Helms J, Murnane T.** 1967. Viruses recovered from laboratory dogs with respiratory disease. Proc Soc Exp Biol Med **126:**140–145. http://dx.doi.org/10.3181/00379727-126-32386.
11. **Rosenberg FJ, Lief FS, Todd JD, Reif JS.** 1971. Studies of canine respiratory viruses. I. Experimental infection of dogs with an SV5-like canine parainfluenza agent. Am J Epidemiol **94:**147–165.
12. **Cornwell HJ, McCandlish IA, Thompson H, Laird HM, Wright NG.** 1976. Isolation of parainfluenza virus SV5 from dogs with respiratory disease. Vet Rec **98:**301–302. http://dx.doi.org/10.1136/vr.98.15.301.
13. **McCandlish IA, Thompson H, Cornwell HJ, Wright NG.** 1978. A study of dogs with kennel cough. Vet Rec **102:**293–301. http://dx.doi.org/10.1136/vr.102.14.293.
14. **Azetaka M, Konishi S.** 1988. Kennel cough complex: confirmation and analysis of the outbreak in Japan. Nippon Juigaku Zasshi **50:**851–858. http://dx.doi.org/10.1292/jvms1939.50.851.
15. **Chladek DW, Williams JM, Gerber DL, Harris LL, Murdock FM.** 1981. Canine parainfluenza-Bordetella bronchiseptica vaccine immunogenicity. Am J Vet Res **42:**266–270.
16. **Kontor EJ, Wegrzyn RJ, Goodnow RA.** 1981. Canine infectious tracheobronchitis: effects of an intranasal live canine parainfluenza-Bordetella bronchiseptica vaccine on viral shedding and clinical tracheobronchitis (kennel cough). Am J Vet Res **42:**1694–1698.
17. **He B, Paterson RG, Ward CD, Lamb RA.** 1997. Recovery of infectious SV5 from cloned DNA and expression of a foreign gene. Virology **237:**249–260. http://dx.doi.org/10.1006/viro.1997.8801.
18. **Phan SI, Chen Z, Xu P, Li Z, Gao X, Foster SL, Teng MN, Tripp RA, Sakamoto K, He B.** 2014. A respiratory syncytial virus (RSV) vaccine based on parainfluenza virus 5 (PIV5). Vaccine **32:**3050–3057. http://dx.doi.org/10.1016/j.vaccine.2014.03.049.
19. **Chen Z, Zhou M, Gao X, Zhang G, Ren G, Gnanadurai CW, Fu ZF, He B.** 2013. A novel rabies vaccine based on a recombinant parainfluenza virus 5 expressing rabies virus glycoprotein. J Virol **87:**2986–2993. http://dx.doi.org/10.1128/JVI.02886-12.
20. **Chai Q, He WQ, Zhou M, Lu H, Fu ZF.** 2014. Enhancement of blood-brain barrier permeability and reduction of tight junction protein expression are modulated by chemokines/cytokines induced by rabies virus infection. J Virol **88:**4698–4710. http://dx.doi.org/10.1128/JVI.03149-13.
21. **Vos A, Muller T, Schuster P, Selhorst T, Wenzel U.** 2001. Maternal immunity against rabies in raccoon dogs. Acta Vet Hung **49:**291–294. http://dx.doi.org/10.1556/AVet.49.2001.3.5.
22. **Aghomo HO, Oduye OO, Rupprecht CE.** 1990. The serological response of young dogs to the Flury LEP strain of rabies virus vaccine. Vet Res Commun **14:**415–425. http://dx.doi.org/10.1007/BF00343220.
23. **Young DF, Randall RE, Hoyle JA, Souberbielle BE.** 1990. Clearance of a persistent paramyxovirus infection is mediated by cellular immune responses but not by serum-neutralizing antibody. J Virol **64:**5403–5411.
24. **Tompkins SM, Lin Y, Leser GP, Kramer KA, Haas DL, Howerth EW, Xu J, Kennett MJ, Durbin RK, Durbin JE, Tripp R, Lamb RA, He B.** 2007. Recombinant parainfluenza virus 5 (PIV5) expressing the influenza A virus hemagglutinin provides immunity in mice to influenza A virus challenge. Virology **362:**139–150. http://dx.doi.org/10.1016/j.virol.2006.12.005.
25. **Li Z, Mooney AJ, Gabbard JD, Gao X, Xu P, Place RJ, Hogan RJ, Tompkins SM, He B.** 2013. Recombinant parainfluenza virus 5 expressing hemagglutinin of influenza A virus H5N1 protected mice against lethal highly pathogenic avian influenza virus H5N1 challenge. J Virol **87:**354–362. http://dx.doi.org/10.1128/JVI.02321-12.

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