Limited Protection Conferred by a DNA Vaccine against a Lethal Pseudorabies Virus Infection at Day 5 Postvaccination

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No pseudorabies virus (PRV)-specific neutralizing or immunoglobulin G1-type antibodies were detected in sera 5 days after injection of a DNA vaccine against PRV infection in pigs. PRV-stimulated peripheral blood mononuclear cells produced gamma interferon mRNA in vitro. Two out of five pigs recovered from lethal PRV infection without attenuation of nasal viral excretion.

Infection of swine with the swine alpha herpesvirus pseudorabies virus (PRV) causes Aujeszky’s disease, a serious illness with high morbidity and mortality leading to severe losses in the pig industry. Various vaccination strategies based on modified live or inactivated vaccines are employed to control Aujeszky’s disease. DNA vaccination provides an alternative to conventional vaccination and is reported to efficiently protect swine, mice, or rabbits against PRV infection 21 to 28 days after plasmid injection (8–10, 14, 18, 30). These ideal experimental conditions are not usually encountered in practice, since it is not known when vaccinated animals will be exposed to viral pathogens. Pigs can be infected soon after vaccination.

The capacity of conventional inactivated or live attenuated vaccines to induce early protection has been described in several animal models of infection. For example, pigs are protected against classical swine fever virus (CSFV) infection 6 days after vaccination (25). Cattle are protected against bovine herpesvirus-1 (BHV-1) infection 3 to 5 days after vaccination (2, 16, 19, 26) and against foot-and-mouth disease virus infection 7 days after vaccination (12, 22). Early protection has also been described for DNA vaccination against viral hemorrhagic septicemia in rainbow trout (20, 24).

We investigated the ability of a single DNA vaccine injection to induce early immune and protective responses against PRV infection in swine. As reported above, different animals, including pigs, were protected against various viral infections 3 to 7 days after vaccination. We selected the minimal period (5 days) needed to confer protection after vaccination in these different studies. The DNA vaccine consisted of the 3 plasmids individually encoding PRV-gB, -gC and -gD, already used successfully in our laboratory (5, 8). Groups of 5 or 6 specific-pathogen-free pigs, housed and treated in accordance with local regulations (Direction des Services Vétérinaires des Côtes d’Armor, France), were injected in the neck with 600 µg of DNA vaccine or empty pcDNA3 5 or 21 days before challenge with 105 50% tissue culture infective doses (TCID50) of the virulent NIA3 PRV strain (kindly provided by J.C. Audonnet, Merial, Lyon, France). Pigs were 11 weeks old, with a mean weight of 38.3 ± 6.2 kg, when challenged.

Titers of serum anti-PRV immunoglobulin G1 (IgG1)-type antibodies, markers of a humoral immune response (23), and neutralizing antibodies (NAb) were determined as previously described (5, 7, 8). These antibodies were not detected 5 days after vaccination and were produced at low levels 7 days after PRV challenge in the group vaccinated 5 days before PRV challenge (Fig. 1 and 2). In comparison, specific anti-PRV IgG1 and NAb (titers of 1:3 and 1:6) were produced in all pigs and in 2 out of 6 pigs, respectively (Fig. 1 and 2). The levels of antibodies increased after PRV challenge.

Levels of porcine gamma interferon (IFN-γ) mRNA, a key mediator for cytotoxic T cells (25, 31), and interleukin-4 (IL-4) mRNA, known to favor a B-cell response (31), were determined in PRV-restimulated peripheral blood mononuclear cells (PBMCs) by quantitative reverse transcriptase PCR as previously described (5, 7). IFN-γ mRNA was produced 5 and 21 days after DNA vaccine injection (Fig. 3A) and was strongly increased in both groups 7 days after challenge. IL-4 mRNA was not detected 5 days after DNA vaccine injection, but low levels were produced at 21 days (Fig. 3B). PRV-stimulated PBMCs from animals of both vaccinated groups produced similar levels of IL-4 mRNA 7 days after PRV challenge (Fig. 3B).

The IFN-γ results suggest that a cellular immune response against PRV is detected as early as 5 days after DNA vaccination. This was also reported 5 days after immunization of cattle against BHV-1 (32) and 6 days after vaccination of pigs against CSFV (25). The levels of IL-4 and PRV antibodies indicate that a humoral immune response was not observed 5 days postvaccination and required between 5 and 21 days to develop.

All pigs injected with empty pcDNA3 died after PRV challenge, lost weight between the day of challenge and day of

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death, developed high fever as early as day 2 postchallenge, and presented mild to severe nervous symptoms (scores were 0, 1, and 2 for no, mild, or severe symptoms, respectively) (Table 1). The levels of viral excretion, determined from swabs of nasal fluid samples as previously described (17, 27), were around 6 and 7.5 log_{10} TCID_{50}/g of nasal swab 2 and 5 days post-PRV challenge, respectively (Table 1). The observed severity of the PRV challenge in the nonvaccinated groups may explain why all pigs in the group vaccinated 21 days before PRV challenge presented mild to severe nervous symptoms and why 2 out of 6 pigs succumbed (Table 1). In these conditions, DNA vaccination 5 days before PRV challenge permitted the survival of 2 out of 5 pigs but did not reduce high fever at day 2 or nasal viral excretion, contrary to the results for the group vaccinated 21 days before challenge (Table 1).

In conclusion, a severe PRV challenge (10^6 TCID_{50}), which provoked the death of all the unvaccinated pigs, was partially controlled in the group vaccinated 5 days before challenge, when a cellular, but not humoral, immune response was present. Cell-mediated immunity is important for early protec-
tion against PRV infection (28, 33) and CSFV infection (25) in swine, against BHV-1 infection in cattle (15, 26), and against viral hemorrhagic septicemia infection in fish (1). Protection was induced by a live attenuated or inactivated vaccine or a protein E-negative vaccine for infectious bovine rhinotracheitis (28, 33) and CSFV infection (25) in pigs. Bovine herpesvirus-1 vaccination against experimental bovine herpesvirus-1 and Pasteurella haemolytica respiratory tract infection: onset of protection. Am. J. Vet. Res. 43:1776–1780.

4. Kaasbøe, M. J., and J. T. van Oirschot. 1996. Early immunity induced by a live gE-negative bovine herpesvirus 1 marker vaccine. Vet. Microbiol. 53:191–197.

5. Kärber, G. 1931. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmacol. 162:480–483.

6. Laval, F., R. Paillet, S. Bollard, L. Fischer, J. C. Audonnet, C. Andreoni, and V. Juillard. 2002. Quantitative analysis of the antigen-specific IFNgamma + T cell response in convalescent outbred pigs: kinetics and duration of the DNA-induced IFNgamma + CD8+ T cell response. Vet. Immunol. Immunopathol. 90:191–201.

7. Makoschey, B., and G. M. Keil. 2003. DNA vaccination against viral haemorrhagic septicaemia (VHS) in rainbow trout: size, dose, route of injection and duration of protection—early protection correlates with Mx expression. Fish Shellfish Immun. 15:39–50.

8. Naumynck, H. J., V. Zonnekeyn, and M. B. Pensaert. 1997. Virological protection of sows upon challenge with Aujeszky’s disease virus after multiple vaccinations with attenuated or inactivated vaccines. Zentbl. Vetmed. B 44:609–615.

9. Pacheco, J. M., M. C. Brum, M. P. Moraes, W. T. Golde, and M. J. Grubman. 2003. Protection of sows upon challenge with Aujeszky’s disease virus confers complete clinical protection in 7 days and partial protection in 4 days: use in emergency outbreak response. Vaccine 23:5775–5782.

10. Haagmans, B. L., E. M. A. van Rooij, M. Dubelaar, T. G. Kimman, M. C. Horzinek, V. E. C. J. Schijns, and A. T. J. Bianchi. 1999. Vaccination of pigs against pseudorabies virus with plasmid DNA encoding glycoprotein D. Vaccine 17:1264–1271.

11. Gerds, V., A. Jöns, B. Makoschey, N. Visser, and C. Mettenleiter. 1997. Protection of pigs against Aujeszky’s disease by DNA vaccination. J. Gen. Virol. 78:2139–2146.

12. Bolin, S. E., P. O. Thomas, and R. F. Marshall. 1975. Early protection against infectious bovine rhinotracheitis with intramuscularly administered vaccine. Proc. Am. Assoc. U.S. Anim. Health Assoc. 1975:50–60.

13. De Bruin, M. G., Y. E. De Visser, T. G. Kimman, and A. T. Bianchi. 2005. Effective protection of pigs against lethal pseudorabies virus infection in pigs. Vaccine 23:1732–1741.

14. Gerdts, V., A. Jöns, B. Makoschey, N. Visser, and C. Mettenleiter. 1997. Protection of pigs against Aujeszky’s disease by DNA vaccination. J. Gen. Virol. 78:2139–2146.

15. Goede, W., J. M. Pacheco, H. Duque, T. Doel, B. Penfold, G. S. Ferman, D. R. Gregg, and L. L. Rodriguez. 2005. Vaccination against foot-and-mouth disease virus confers complete clinical protection in 7 days and partial protection in 4 days: use in emergency outbreak response. Vaccine 23:5775–5782.

16. Todd, J. D., F. J. Volence, and J. M. Paton. 1971. Intra nasal vaccination against infectious bovine rhinotracheitis: studies on early onset of protection and use of the vaccine in pregnant cows. J. Am. Vet. Med. Assoc. 159:1370–1374.
27. Vannier, P., E. Hutet, E. Bourgueil, and R. Cariolet. 1991. Level of virulent virus excreted by infected pigs previously vaccinated with different glycoprotein deleted Aujeszky’s disease vaccines. Vet. Microbiol. 29:213–223.
28. van Rooij, E. M., M. G. de Bruin, Y. E. de Visser, W. G. Middel, W. J. Boersma, and A. T. Bianchi. 2004. Vaccine-induced T cell-mediated immunity plays a critical role in early protection against pseudorabies virus (suid herpes virus type 1) infection in pigs. Vet. Immunol. Immunopathol. 99:113–125.
29. van Rooij, E. M., H. L. Glansbeek, L. A. Hilgers, E. G. te Lintelo, Y. E. de Visser, W. J. Boersma, B. L. Haagmans, and A. T. Bianchi. 2002. Protective antiviral immune responses to pseudorabies virus induced by DNA vaccination using dimethyldioctadecylammonium bromide as an adjuvant. J. Virol. 76:10540–10545.
30. van Rooij, E. M. A., B. L. Haagmans, Y. E. de Visser, M. G. M. de Bruin, W. Boersma, and A. T. J. Bianchi. 1998. Effect of vaccination route and composition of DNA vaccine on the induction of protective immunity against pseudorabies infection in pigs. Vet. Immunol. Immunopathol. 66:113–126.
31. Wood, P. R., and H. F. Seow. 1996. T cell cytokines and disease prevention. Vet. Immunol. Immunopathol. 54:33–44.
32. Woolums, A. R., L. Siger, S. Johnson, G. Gallo, and J. Conlon. 2003. Rapid onset of protection following vaccination of calves with multivalent vaccines containing modified-live or modified-live and killed BHV-1 is associated with virus-specific interferon gamma production. Vaccine 21:1158–1164.
33. Zuckermann, F. A. 2000. Aujeszky’s disease virus: opportunities and challenges. Vet. Res. 31:121–131.