

THE EFFECTS OF VACCINATION ON THE IMMUNE RESPONSE IN PSEUDORABIES VIRUS CHALLENGED SWINE

ROBERTO ALVA V.,* ROBERT D. GLOCK, JOHN P. KLUGE, HOWARD T. HILL

DEPARTMENT OF VETERINARY PATHOLOGY, COLLEGE OF VETERINARY MEDICINE, IOWA STATE UNIVERSITY AMES IOWA 50011

Introduction: The phenomenon of cell-mediated immunity can be evaluated by the effects of specific antigens on lymphocytes *in vitro*.¹ The blast transformation test measures the *in vitro* proliferative response of previously sensitized T cells to specific antigen. The lymphoproliferative response of swine lymphocytes to pseudorabies virus (PRV) has been recently reported.^{2,3}

Objectives: The objectives of this study were to determine if the development of cellular immunity is useful in predicting response to challenge and to determine the degree of protection due to vaccination.

Materials and Methods: Ninety-three-seven-week castrated male pigs were utilized. Animals were free of clinical atrophic rhinitis and of serologic evidence of pseudorabies (PR), transmissible gastroenteritis and leptospirosis. The pigs were divided in groups number I,II,III,IV,V,VI, and VII and challenged with PRV at 1,3,5,8,10,12, and 14 months after vaccination respectively. Groups I,II,III, and IV consisted of 4 pigs vaccinated with a modified live PRV (MLPRV),^a vaccine, 4 vaccinated with an inactivated PRV (IPRV)^b vaccine, and 4 injected with a placebo solution (PI) of sterile distilled water. Groups V,VI, and VII consisted of 5 pigs of each category (5 MLPRV, 5 IPRV, and 5 PI). All pigs were vaccinated at 9 weeks of age. Level of cell mediated immunity (CMI) was measured by the lymphocyte transformation test (LTT) *in vitro*. The LTT was measured in half the animals before challenge and at 6 days after challenge (AC), and in the other half before challenge and at 6,11,36, and 60 days AC. Humoral immunity was determined by the microtitration serum neutralization test (MSNT). The MSNT was conducted for 16 months after vaccination before challenge and at 7 and 60 days AC. Serums from groups I,II,III, and IV were tested simultaneously while serums from groups V,VI, and VII were tested separately at monthly intervals. Virus isolation from brain and spleen and fluorescent antibody test on lungs, tonsils and trigeminal ganglia were conducted on pigs killed at 7 and 60 days AC. Virus co-culture from trigeminal ganglia were conducted in pigs killed at 60 days AC. Bacteriologic examination was conducted on all major organs. Four ml. of an Iowa strain of PRV at the 10th and 11th tissue culture passage containing 1.6×10^6 and 5×10^6 plaque forming units respectively was used as the challenge inoculum. The inoculum was administered deeply into the nasal cavity. The PRV antigen used in the LTT was frozen, thawed, sonicated, precipitated and concentrated by centrifugation and addition of polyethylene glycol (Mol. wt. 20,000). For the LTT blood was collected into siliconized tubes with heparin. Mononuclear cells were separated and a dilution of 4.7×10^6 cells/ μ l. prepared. One hundred and fifty μ l of cells were added to each well of a 96 well U bottom microtiter plate. The PRV antigen was diluted 1 to 80 and 50 μ l of it added to each well. Control wells received 50 μ l of RMPI 1640 media, while positive control wells received 10 μ g of phytoemagglutinin P (PHA-P).³ H thymidine was added and the cells harvested onto glass fiber filter paper. Samples were counted in a liquid scintillation counter. Results were expressed in counts per minute (CPM) and stimulation ratios (division of CPM of the cells containing the PRV antigen by CPM of cells containing the control media RMPI 1640). The LTT was considered positive when stimulation ratios (SR) were higher than 3 to 1. By using the student T test the SR between PI,IPRV, and MLPRV vaccinated pigs were compared. The techniques used for the MSNT, virus co-culture, virus isolation and fluorescent antibody tests were the same as previously described.^{4,5} The MSNT was considered positive at the 1:2 dilution or great-

er. Fluorescent antibody, virus isolation, and virus co-culture tests were scored as positive or negative.

Results: The SR after vaccination before challenge were lower than 3 to 1 in the great majority of the pigs. The response was first detected and reached the highest value at 6 days AC and remained high for 60 days AC. The MLPRV vaccinated pigs had the overall highest SR (9 to 1) AC followed by the IPRV (6.6 to 1) and PI vaccinated (4.6 to 1) respectively. The highest percent of seropositive results in the IPRV vaccinated was 84% (27 out of 32), while the highest seropositive results for the MLPRV vaccinated was 90% (28 out of 31). Only 21% (3 out of 14) PI injected pigs seroconverted at 7 days AC, while the IPRV and MLPRV vaccinated did so at 100% (13 out of 13) and 85% (12 out of 14) levels respectively. At 60 days AC 86% (12 out of 14) of the pigs in each category seroconverted. The MLPRV vaccinates had the highest antibody titers followed by the IPRV vaccinated and PI injected. Vaccinated pigs in groups I,III, and IV had the highest serum neutralization titers while groups II,V,VI and VII had the lowest. In the PI injected, PRV was isolated in 43% (6 out of 14) of the pigs at 7 days AC, while at 60 days virus was found in only 7% (1 out of 14). In the IPRV vaccinated pigs virus was isolated in 7% (1 out of 14) pigs at 7 days AC, no virus was isolated at 60 days AC. In the MLPRV vaccinated, virus was detected in 7% (1 out of 14) at 7 and 60 days respectively. The fluorescent antibody test was positive in 50% (7 out of 14) and in 7% (1 out of 14) of the lungs and trigeminal ganglia at 7 days AC. Thirty six percent (5 out of 14) of MLPRV vaccinated were positive at 7 days AC, while all were negative at 60. Virus co-culture and bacteriologic examinations were negative.

Conclusions: The IPRV vaccine utilized in this experiment induced a primary humoral immune response detectable for 8 months after vaccination, while the MLPRV vaccine induced the same response for 13 months after vaccination. The protection conferred by vaccination was attributed to a primary humoral immune response following vaccination and to a secondary humoral and cellular immune response AC. Secondary levels of humoral and cellular immunity AC were higher in vaccinated pigs when compared to non-vaccinated. The LTT *in vitro* is useful in predicting early response to challenge specially in non-vaccinated pigs in which the primary humoral immune response develops within 7 to 8 days AC. Virus isolation attempts from vaccinated pigs may give negative results.

Selected References:

1. Bloom, B.R.: *In Vitro Methods in Cell Mediated and Tumor Immunology*, New York, Academic Press. (1976)
2. Rottinghaus, A.A.; Hill, H.T.; Meetz, M.C.: *Am. Assoc. Vet. Lab. Diag. Annual Proc.* 21: 57--66 (1978)
3. Wittmann, G.; Bartenbach, G.; Jakubik, J.: *Arch. Virol.* 50: 215-222 (1976)
4. Hill, H.T.; Crandell, R.A.; Kanitz, CHL.; McAdaragh, S.; Seawright, G.L.; Solorzano, R.F.; Steward, W.C.: *Am. Assoc. Vet. Lab. Diag. Annual Proc.* 20: 375-386 (1977)
5. Beran, G.W.; Davies, B.E.; Arambulo, P.V.; Will, L.A.; Hill, H.T.; Roch, D.L.: *J. Am. Vet. Med. Assoc.* 10: 990-1000 (1980)

^aNorden Laboratories Inc., Lincoln, Nebraska 68501 USA.

^bSalisbury Laboratories, Charles City, Iowa 50616 USA.