Characterization of Immune Ontogeny of Young Swine to Porcine Circovirus Type 2 Infection

R. Pogranichnyy, graduate assistant,
K.-J. Yoon, assistant professor,
Department of Veterinary Diagnostic and Production Animal Medicine
and
S. L. Swenson, collaborator,
National Veterinary Services Laboratories

ASL-R1697

Summary and Implications

A longitudinal study was conducted to characterize the humoral immune response of pigs to porcine circovirus type 2 (PCV2), the postulated causative agent for postweaning multisystemic wasting syndrome (PMWS). Eight-week-old cesarean-derived, colostrum-deprived (CD/CD) pigs were inoculated with a purified isolate of PCV2 and kept for 35 days post-inoculation (PI). Serum samples were collected from all pigs on day 0 and, thereafter, every 7 days until termination of the study. Naïve young pigs were shown to be susceptible to PCV2. PCV2-specific antibodies were detected by an indirect fluorescent antibody test at day 7 PI and after, whereas neutralizing antibodies were not detected until day 28 PI. Western immunoblot analysis of the sera demonstrated three virus-specific proteins with molecular mass of 28, 28.5 and 35 kD. By comparing the appearance of antibody with protein specificity of antibody response, the 28-kD protein was highly immunogenic and specific for PCV2, suggesting that the 28-kD protein may provide the antigenic basis for the development of diagnostic tests for the detection of PCV2-specific antibody. Other two proteins may be associated with virus neutralization.

Introduction

Porcine circovirus (PCV) is a small, negative-sense, single-stranded DNA virus (11). The PCV was first detected as a noncytopathic viral contaminant of a continuous pig kidney cell line PK-15 (4,17). Several independent serologic surveys of swine in Canada, Germany, United States, and other countries revealed that the virus is highly prevalent in domestic swine (2,3,4,5,8,10,16). Antibodies reactive to PCV also were detected in humans, mice, and cattle (15). Despite of high serologic prevalence in swine, the virus was not previously associated with specific disease in pigs (1,6,9,16). No clinical disease or pathological changes have been demonstrated in pigs experimentally infected with PCV (1).

Recently, a disease termed PMWS, which is characterized by progressive weight loss in pigs at 4–16 weeks of age, has emerged in swine industry (3,9). In 1996 the syndrome was described in swine herds with "high health" status in Canada as a chronic and sometimes protracted disease (3,14). Shortly after, the syndrome was reported in the United States, Europe, and Asia (2).

A circovirus that is genetically and antigenically distinct from the PCV originally found in a PK-15 cell line has been isolated and/or detected from pigs affected by PMWS (2,13) This field isolate was designated PCV2 as opposed to PCV type 1 (PCV1) for the PK-15 cell contaminant (7,12). Although the virus was recovered or detected from affected pigs, Koch's postulates have not been fulfilled. The virus has not been characterized well. The following study was conducted to characterize the immunological response of pigs to PCV2 and determine the role of viral protein in immune response.

Materials and Methods

Five, 5-week-old CD/CD pigs were obtained and housed in the biosafety level three animal facility at the National Animal Disease Center, USDA/ARS, Ames, IA. Prior to inoculation, pigs were tested twice for the presence of serum antibodies against PCV types 1 and 2 by an indirect fluorescent antibody (IFA) test at a 2-week interval and confirmed negative for both types of PCV. The pigs also were tested for serum antibodies against major swine pathogens. At approximately 8 weeks of age, each animal was inoculated intranasally (1 ml/naris) and intramuscularly (2 ml/pig) with a purified isolate of PCV2 recovered from a pig diagnosed with PMWS at a rate of 10^4 $TCID_{50}$/ml. Then all pigs were monitored for 5 weeks PI. Pigs were bled on the day of inoculation and every 7 days thereafter during the study period. Serum was separated and stored at -80° C until tested. Viremia was monitored by a polymerase chain reaction (PCR) assay developed in our laboratory. Antibody response to PCV2 and PCV1 was assessed using IFA test and virus neutralization (VN) assay using protocols established in our laboratory. The protein specificity of antibody response was characterized with Western immunoblotting.

Result and Discussion

All inoculated pigs remained normal in their appearance and behavior throughout the study period. In contrast, as determined by detection of the presence of PCV2 DNA in serum by PCR, all pigs became viremic after inoculation. Viremia was detected in all pigs on days seven and 14 PI and in two of the five pigs on day 21 PI, demonstrating that naïve pigs are susceptible to PCV2.

The humoral immune response of the pigs to PCV2 is summarized in Figure 1. Prior to and on the day of challenge none of the five pigs had antibody to PCV2 as well as PCV1 as measured by IFA and VN tests. After challenge all pigs

seroconverted to PCV2. Virus-specific IgG antibody was initially detected by IFA test at 14 days PI, ranging 1:80 to 1:640, and continued to increase to the mean titer of 1:1536, ranging from 1:640 to 1:2560, by the end of the study. Neutralizing antibody against PCV2, however, was first detected at 4 weeks PI and present at the termination of the study. The titer was 1:8.

Interestingly, as neutralizing antibody to PCV2 appeared, IFA antibody that cross-reacted with PCV1 also was detected in all pigs. Yet antibody titers to PCV1 were significantly lower, ranging 1:160 to 1:320, at the end of the study. In comparison, when serum samples collected at the end of study were tested by the IFA and SN tests against four other field isolates of PCV2, no significant difference in antibody titers to individual isolates was detected compared with that against homologous isolate. It suggests no significant antigenic difference among PCV2 isolates**.**

Under reducing condition, three polypeptides with apparent molecular mass of 28, 28.5 and 35 kD, respectively, were demonstrated to be present in PCV2 with serum samples collected at the end of the study (Figure 2B). Among these proteins, the 35-kD protein also was shown to be associated with PCV1 as well, indicating that this protein may be responsible for the cross-reactivity between PCV1 and PCV2 that was observed in our study without apparent introduction of PCV1 to the pigs. Previous serological surveys with PK-15 contaminated PCV, i.e., PCV1, demonstrated that the virus is highly prevalent in swine. Such a high prevalence may have been attributed to the cross-reactivity between PCV1 and PCV2. If that is the case, PCV2 may have been present in the swine population for a longer period of time than thought, which is the subject of further study.

Temporal viral protein specificity of immune response that was characterized by Western immunoblot analysis is summarized in Figure 2A. Antibodies reactive to the 28-kD protein were first detected in pigs at 14 days PI and persisted to the end of the study. Assuming that higher intensity of reaction to a protein in the Western immunoblotting is a reflection of higher amount of antibody in serum under the same running condition, level of antibodies to the 28-kD protein in sera at a given dilution was always much higher than antibodies to the other two proteins. These results indicate that the 28-kD protein may provide the antigenic basis for the development of diagnostic tests for the detection of PCV2-specific antibody.

Antibodies to the 28.5-kDand 35-kD proteins were first detected at day 21 PI and continued to be detected by the end of the study. The appearance of antibodies to the 28.5-kD and 35-kD proteins coincided with the detection of neutralizing antibody in serum, suggesting that these two proteins are associated with the induction of neutralizing activity. Although it is not certain which of these proteins is mainly associated with virus neutralization, the 35-kD protein appears to contain major neutralizing epitope(s) based on the difference in intensity of antibody response to these proteins detected by Western immunoblotting. As shown in Figure 2B, antibody response to the 35-kD protein was very weak on day 21 PI and became stronger at day 28 PI when detectable VN antibody was present, whereas much more intensive reaction was already detected with 28.5-kD protein at day 21 PI when no neutralizing antibody was detected. However, the potential role that antibodies specific for 28.5-kD and 35-kD proteins may play in viral neutralization requires further study.

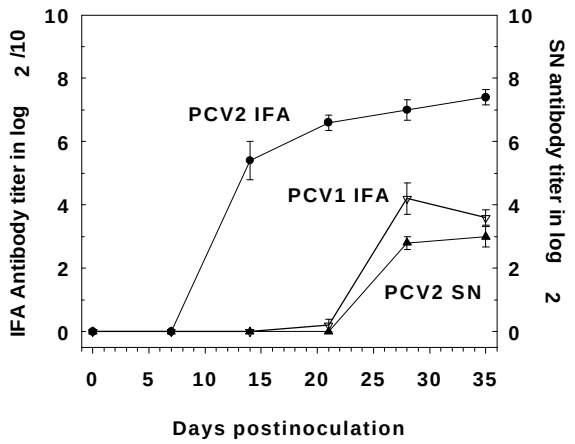

Figure 1. Antibody response of young pigs to porcine virus type 2 (PCV2) infection as determined by an indirect fluorescent antibody (IFA) test and serum neutralization (SN) test.

References

1. Allan, G.M., F. McNeilly, J.P. Cassidy, G.A. Reilly, B. Adair, W.A. Ellis, and M.S. McNulty. 1995. Pathogenesis of porcine circovirus: experimental infections of colostrum deprived piglets and examination of pig foetal material. Vet. Microbiol. 44: 49–64.
2. Allan, G.M., F. McNeilly, S. Kennedy, B. Daft, E.G. Clarke, J.A. Ellis, D.M. Haines, B.M. Meehan, and B.M. Adair. 1998. Isolation of porcine circovirus-like viruses from pigs with a wasting disease in the USA and Europe. J. Vet. Diagn. Invest. 10: 3–10.
3. Clark, E.G. 1997. Post-weaning multisystemic wasting syndrome. pp. 499–501. Proc. Am. Assoc. Swine Pract.
4. Dulac, G.C. and A. Afshar. 1989. Porcine circovirus antigens in PK-15 cell line (ATCC CCL-33) and evidence of antibodies to circovirus in Canadian pigs. Can. J. Vet. Res. 53: 431–43.

5. Edwards, S. and J.J. Sands. 1994. Evidence of circovirus infection in British pigs. Vet. Rec. 134: 680–681.
6. Ellis, J., L. Hassard, E. Clark, J. Harding, G. Allan, P. Willson , J. Strokappe, K. Martin, F. McNeilly, B. Meehan, D. Todd, and D. Haines. 1998. Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. Can. Vet. J. 39: 44-51.
7. Hamel, A.L., L.L. Lin, and G.P. Nayar. 1998. Nucleotide sequence of porcine circovirus associated with postweaning multisystemic wasting syndrome in pigs. J. Virol. 72: 5262–5267.
8. Harding, J. 1997. Post-weaning multisystemic wasting syndrome. p. 503. Proc. Am. Assoc. Swine Pract.
9. Harding, J.S., E.G. Clark, J.H. Strokappe, P.I. Willson, and J.A. Ellis. 1998. Postweaning multisystemic wasting syndrome: Epidemiology and clinical presentation. Swine Health Prod. 6: 249–254.
10. Kiupel, M., G.W. Stevenson, S.K. Mittal, E.G. Clark, and D.M. Haines. 1998. Circovirus-like viral associated disease in weaned pigs in Indiana. Vet. Pathol. 35: 303–307.
11. Mankertz, J., H.J. Buhk, G. Blaess, and A. Mankertz. 1998. Transcription analysis of porcine circovirus (PCV). Virus Genes 16: 267–276.
12. Meehan, B.M., F. McNeilly, D. Todd, S. Kennedy, V.A. Jewhurst, J.A. Ellis, L.E. Hassard, E.G. Clark, D.M. Haines, and G.M. Allan. 1998. Characterization of novel circovirus DNAs associated with wasting syndromes in pigs. J. Gen. Virol. 79: 2171–2179.
13. Morozov, I., T. Sirinarumitr, S.D. Sorden, P.G. Halbur, M.K. Morgan, K.J. Yoon, and P.S. Paul. 1998. Detection of a novel strain of porcine circovirus in pigs with postweaning multisystemic wasting syndrome. J. Clin. Microbiol. 36: 2535–2541.
14. Nayar, G.P., A. Hamel, and L. Lin. 1997. Detection and characterization of porcine circovirus associated with postweaning multisystemic wasting syndrome in pigs. Can. Vet. J. 38: 385–386.
15. Tischer, I., L. Bode, D. Peters, S. Pociuli, and B. Germann. 1995. Distribution of antibodies to porcine circovirus in swine populations of different breeding farms. Arch. Virol. 140: 737–743.
16. Tischer, I., W. Mields, D. Wolff, M. Vagt, and W. Griem. 1986. Studies on epidemiology and pathogenicity of porcine circovirus. Arch. Virol. 91: 271–276.
17. Tischer, I., R. Rasch, and G. Tochtermann. 1974. Characterization of papovavirus-and picornavirus-like particles in permanent pig kidney cell lines. Zentralbl. Bakteriol. [A] 226: 153–167.

Acknowledgment

This study was supported in part by funding from Iowa Healthy Livestock Initiative Research Grant and Iowa Livestock Health Advisory Council.

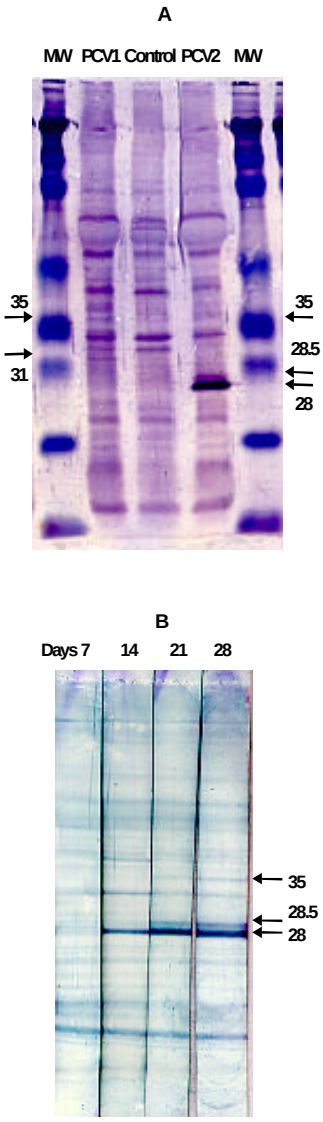

Figure 2. Western immunoblot analysis of sera collected from pigs inoculated with porcine circovirus type 2 (PCV2). A, protein composition of PCV2. B, protein specificity of antibody response in the pigs. Numbers on the top of (B) refer to days PI. Numbers on side refer to approximate molecular mass of reactive proteins in kilodaltons.