Correlation of cell-mediated immunity against porcine reproductive and respiratory syndrome virus with protection against reproductive failure in sows during outbreaks of porcine reproductive and respiratory syndrome in commercial herds

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Objective—To determine whether cell-mediated immunity against porcine reproductive and respiratory syndrome (PRRS) virus is correlated with protection against reproductive failure in sows during clinical outbreaks of PRRS in commercial herds.

Design—Outbreak investigation in 4 swine breeding herds.

Animals—97 sows.

Procedures—On each farm, blood samples were collected from sows with clinical signs (abortion or increased fetal death; case sows) and from clinically normal sows (control sows). The intensity of the cell-mediated immune (CMI) response was determined by use of an interferon-γ enzyme-linked immunospot (ELISPOT) assay. Multiple logistic regression analyses and *t* tests were used to compare ELISPOT assay values between case and control sows. Multiple linear regression was used to investigate associations between cell-mediated immunity and the magnitude of clinical signs.

Results—In 2 farms, case sows had lower ELISPOT assay values than control sows. A negative association between the intensity of the CMI response and the number of pigs born dead per litter was detected on 1 farm. In 1 farm, no association was detected between the intensity of the CMI response and protection against reproductive failure.

Conclusions and Clinical Relevance—Evidence that a strong CMI response was correlated with protection against clinical PRRS was detected in 3 of 4 farms. However, farms and sows within farms varied considerably in their immune responsiveness and in the degree to which they were protected clinically. Increasing cell-mediated immunity within infected herds has the potential to decrease clinical reproductive disease, but only if the sources of intra- and interfarm variation in the intensity of cell-mediated immunity to PRRS virus can be identified. (*J Am Vet Med Assoc* 2005;226:1707–1711)

The nature and extent of clinical signs in swine herds infected with **porcine reproductive and respiratory syndrome virus (PRRSV)** vary widely.[1,2] Within and between herds, some sows infected with PRRSV have abortions[3,4] and fetal death,[3,5-7] whereas others appear clinically unaffected. The reasons for this wide variation are not clear. Differences among viral strains, environmental conditions (including other pathogens), management practices, and host immunity could explain the clinical variability of PRRSV infection in the field.[8,9] In reproductive herds, abortion and fetal death associated with **porcine reproductive and respiratory syndrome (PRRS)** result in decreased numbers of pigs weaned from sow farms that are infected. These clinical outcomes cause important economic losses.[7,10]

Control strategies presently used on infected farms are directed at increasing PRRSV-specific immunity in breeding females to prevent transmission of the virus and reduce the duration of clinical signs.[11-14] These strategies are promising because swine develop specific humoral and **cell-mediated immune (CMI)** responses against PRRSV. The immune response against PRRSV has been found to be protective against repeat challenge with the same virus for at least 604 days after initial infection.[15] Swine develop all major classes of antibodies against PRRSV, and the kinetics of their development have been described.[16,17] Antibodies directed against certain epitopes of glycoprotein 5 have been found to neutralize PRRSV.[18] Swine also develop a specific CMI response to PRRSV, which, although it develops slowly, may provide some degree of clinical protection in controlled settings.[19,20]

During experimental conditions, the adaptive immunity developed against PRRSV is protective against reproductive failure associated with PRRSV infection. Lager et al[21] reported that sows previously infected with PRRSV were at least partially protected against reproductive failure at the time of known exposure, whereas those that were not previously infected were not protected. More specifically, increased quantities of PRRSV-specific neutralizing antibody given prior to exposure have prevented abortion in sows

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Supported by a grant from USDA, Cooperative State Research, Education and Extension Service (proposal No. 2003-02374) and by the Illinois Council for Food and Agricultural Research.

The authors thank Mauricio Villamar and Lynn Collura for assistance.

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exposed to PRRSV.[22] The CMI response to PRRSV appears to be at least equally important, although it is less well understood. **Interferon-γ (INF-γ)**, a cytokine integral to the primary response of the CMI system, blocks the infection of porcine macrophages[23] and other cells[24] by PRRSV. In addition, **INF-γ–secreting (INF-γ-SC)** cells and INF-γ have been detected in pig lungs infected with PRRSV.[25,26] The number of **peripheral blood mononuclear cells (PBMCs)** that secrete INF-γ in response to virus has been associated with the clearance of pseudorabies virus from infected pigs.[27] Although the kinetics of the INF-γ response to PRRSV are slower than the response to pseudorabies virus,[20] some swine nevertheless eventually develop a strong and sustained response.[20] Therefore, it is reasonable that the CMI response plays a role in the protection of swine against PRRSV.

Despite rapid advances in the laboratory, the role of PRRSV-specific immunity in preventing clinical disease in the field is poorly understood. The purpose of the study reported here was to determine whether cell-mediated immunity to PRRSV is correlated with protection against reproductive failure in sows in commercial herds during clinical outbreaks of PRRS. Outbreaks of PRRS in 4 commercial herds are described for which, in addition to routine diagnostic evaluations, data on the PRRSV-specific CMI response of individual sows were also collected. The investigation focused on the role that PRRSV-specific cell-mediated immunity may have in decreasing clinical signs in breeding swine.

Materials and Methods

Study herds and selection of sows—Suspected outbreaks of clinical PRRS were investigated in 4 herds from January 2002 to August 2003. All 4 herds were known to have been infected with PRRSV, and various control measures to decrease clinical signs in the herd had been used. In all 4 herds, the clinical signs of concern were associated with reproductive failure. In addition, growing pigs in all herds had clinical signs of pneumonia.

Farms A and B were chronically infected with PRRSV and were having increased rates of abortion in sows in middle to late gestation. Farms C and D had not been previously infected with PRRSV until 7 and 8 months prior to the outbreak investigations, respectively. Farms C and D were having increased rates of fetal death as evidenced by increased rates of stillborn and mummified piglets at birth.

Farm A was located in the Eastern United States and housed 2,500 swine used for breeding stock only. Offspring from this farm were moved to another site at weaning. The farm had been infected with PRRSV for > 5 years at the time of the investigation, and replacement females were obtained from another farm infected with PRRSV. The farm had a history of using a commercial modified-live virus vaccine; however, no vaccine was being used at the time of sample collection. The farm was having an epidemic of late-term gestation (mean ± SEM, 103.3 ± 1.89 days of gestation) abortions when the investigation was performed. Eighteen sows were chosen for inclusion in the study. Nine of those sows had aborted (case sows) in the 7 days prior to sample collection. Nine sows that had not aborted (control sows) were chosen and matched with aborting sows on the basis of similarity of breeding date (day of gestation) and parity.

Farm B was located in the Midwestern United States and housed approximately 8,500 swine used for breeding and growing stock. The breeding herd consisted of 800 males and females, and replacement females were obtained from within the farm, allowing for natural infection during the growing period. The farm had a history of using various commercial modified-live and killed virus vaccines, but none were used in the 6 months prior to sample collection. The farm was having an epidemic of midterm gestation (77.25 ± 4.77 days of gestation) abortions when the investigation was performed. Twenty-six sows were chosen for the investigation. Thirteen sows that had aborted (case sows) in the 14 days prior to sample collection were chosen for inclusion in the study, as were 13 matched sows that had not aborted (control sows). Sows were matched on the basis of breeding date (day of gestation) and parity.

Farm C was located in the Midwestern United States and housed 1,250 swine used for breeding stock only. Offspring from this farm were moved to another site at weaning. This farm had not been infected with PRRSV until 7 months before the investigation began. At the time of initial infection, the herd had an increased rate of abortions and an increase in the number of stillborn and mummified piglets at birth. There was no history of vaccine use on the farm, and replacement females had not been introduced into the on-site growing facilities, which were infected with PRRSV at the same time as the sow herd, from the initial outbreak until the time of sample collection. A review of farm records suggested that some sows were having litters with expected numbers (1 to 2 pigs) of pigs born dead, whereas others that were housed with the clinically normal sows were having litters in which > 70% of the litter was born dead. Twenty-nine sows from a single weaning cohort of approximately 56 sows that were parity 1 or 2 were chosen randomly for inclusion in the investigation. The total number of pigs born and the number of pigs born dead (still births and mummified fetuses) were recorded from farm records for each sow.

Farm D was located in the Midwestern United States and housed 5,400 swine used for breeding stock only. Offspring from this farm were moved to another site at weaning. This farm had not been infected with PRRSV until 8 months before the investigation began. There was no history of vaccine use on the farm, and replacement females were not exposed to PRRSV until they were naturally infected at 6 months of age, when they were introduced to the onsite isolation facility. At the time of initial infection, the herd had a low number of abortions but had a high rate of fetal mortality (> 15% of pigs born) and neonatal death (> 15% of pigs born alive). A review of the records suggested that some sows were having litters with expected numbers (1 to 2 pigs) of pigs born dead and others that were housed with normal sows were having litters in which 90% to 100% of the litter was born dead. Twenty-four sows were chosen for inclusion in the study. All sows were fourth parity and were chosen from a single farrowing cohort of approximately 260 sows. Twelve of the chosen sows had > 4 pigs born dead per litter (case sows), and 12 of the sows did not have any pigs born dead per litter (control sows). Control and case sows were matched on the basis of the total number of pigs born per litter and by farrowing date.

Sample collection, processing, and analysis—Two 7- to 10 mL samples of blood were collected from each sow via standard sterile jugular venipuncture. The first sample was collected in a serum separator tube[a] and the second in a 10-mL sterile vacuum tube coated with heparin.[b] All samples were placed on ice and transported to the laboratory for analysis within 24 hours of collection. Serum was separated from the serum sample via centrifugation. Serum samples from farm C were analyzed for non-neutralizing antibodies against PRRSV with a commercial ELISA,[c] and a commercial fluorescent focus neutralization test[d] was used to quantify

neutralizing antibodies in samples from farm B. Mononuclear cells were isolated from heparinized blood samples by use of density gradient centrifugation and analyzed for the intensity of the CMI response to PRRSV by use of a PRRSV-specific, INF-γ **enzyme-linked immunospot (ELISPOT)** assay, as previously described.[20] The ELISPOT assay measures the number of PRRSV-specific, INF-γ-SC memory T cells and is expressed as the number of INF-γ-SC cells per 1 million PBMCs.[20] The number of IFN-γ-SC, virus-specific PBMCs has been found to correlate positively with protection against pseudorabies virus.[27]

Statistical analyses—Mean ELISPOT assay values of case and control sows on farms A[e] and B[f] were compared by use of paired and unpaired *t* tests, respectively.[28] To combine data from both farms into a single analysis, a mixed-effects multiple logistic regression model[g] was used in which INF-γ ELISPOT assay values were used to predict the binary outcome of abortion or no abortion and in which random differences between the 2 farms in the proportion of aborting sows were accounted for by use of a single dummy-coded random effects variable.[28]

A linear regression model[h] was used to test the hypothesis that sows with high INF-γ ELISPOT assay counts had low proportions of pigs born dead per litter on farm C.[28] A multivariate model[h] that included the total number of pigs born and proportion of pigs born dead per litter as dependent variables and the INF-γ ELISPOT assay count as the independent variable was also constructed for farm C.[28] The same hypothesis was tested on data from farm D with a paired *t* test[f] because sows were preselected to fall into 1 of 2 groups: case sows (high numbers of dead pigs/litter) and matched control sows (no dead pigs/litter).[28]

Results

Results of the INF-γ ELISPOT assays indicated that CMI responses of case and control sows varied between the 2 farms with increased abortions (mean ± SEM, 210.84 ± 35.25 INF-γ-SC cells/10^6 PBMC vs 121.02 ± 17.48 INF-γ-SC cells/10^6 PBMC). On farm A, sows that aborted had fewer PRRSV-specific, INF-γ-SC cells than did sows that did not abort (127.1 ± 35.4 INF-γ-SC cells/10^6 PBMC vs 303.89 ± 47.3 INF-γ-SC cells/10^6 PBMC); this difference was significant (t = –3.07; P = 0.007). On farm B, 4 sows that had been chosen as controls aborted during the 14 days after sample collection and were therefore considered for the purposes of analysis to be case sows, resulting in 17 case and 9 control sows. Sows on farm B that aborted had a lower number of PRRSV-specific, INF-γ-SC cells than sows that did not abort (108.4 ± 20.0 INF-γ-SC cells/10^6 PBMC vs 144.89 ± 33.7 INF-γ-SC cells/10^6 PBMC), but this difference was not significant (t = –0.42; P = 0.334). There was no significant difference in the geometric mean fluorescent focus neutralization test titers between sows that aborted (48.87) and did not abort (47.03). Results of the combined analysis for both farms indicated that cell-mediated immunity was generally protective; an increased CMI response decreased the risk of a given sow aborting (odds ratio, 0.64; per 50 unit change in the INF-γ ELISPOT assay count; 95% confidence interval, 0.47 and 0.90; χ^2, 5.55; P = 0.02).

On farm C, all sows had non-neutralizing antibodies against PRRSV (sample-to-positive ratio = 1.17 ± 0.13), and the mean INF-α ELISPOT assay count was higher on farm C than on farm D (133.24 ± 19.50 INF-γ-SC cells/10^6 PBMC vs 81.98 ± 13.00 INF-γ-SC cells/10^6 PBMC). The proportion of pigs born dead per litter was negatively associated with the intensity of the CMI response (r^2 = 0.158; P = 0.024; **Figure 1**) on farm C. On farm D, there was no significant difference in PRRSV-specific, INF-γ-SC cells detected between case (89.58 ± 21.08 INF-γ-SC cells/10^6 PBMC) and control sows (73.68 ± 15.21 INF-γ-SC cells/10^6 PBMC; t = –0.61; P = 0.294).

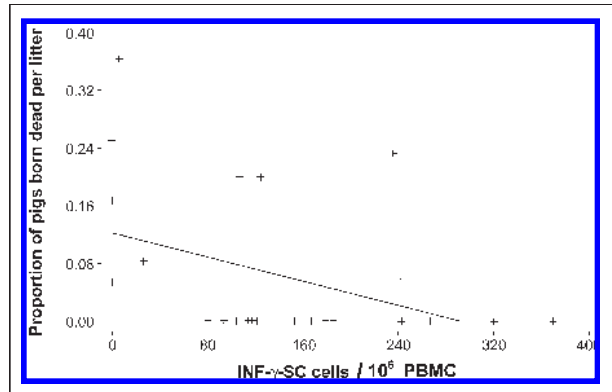

Figure 1—Association between proportion of pigs born dead per litter and number of porcine reproductive and respiratory syndrome virus specific interferon-γ-secreting (IFN-γ-SC) cells from sows (n = 29) having litters in which > 70% of the litter was born dead (r^2 = 0.158; P = 0.024). PBMC = Peripheral blood mononuclear cell.

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Discussion

On farms A and B, aborting sows had lower numbers of PRRSV-specific, INF-γ-SC cells than sows that did not abort. This difference was significant for farm A but not for farm B. However, results of combined data from both farms were significant. The mean number of PRRSV-specific, INF-γ-SC cells of all sows was higher on farm A than on farm B. Therefore, the lack of a significant finding on farm B may have resulted from low cell-mediated immunity overall and consequently low interindividual variation in INF-γ ELISPOT assay counts on that farm. The reasons why these 2 farms differed in this respect are not clear but may have been attributable to different intensities or durations of infection on each farm or from unquantified environmental factors.

On farm C, there was a significant association between PRRSV-specific, INF-γ-SC cells and proportions of pigs born dead per litter. The data from farm C support the hypothesis that PRRSV-specific cell-mediated immunity is at least partially protective against fetal death from PRRSV infection and is in agreement with findings of another study.[6] However, no such association was detected on farm D. Differences between farms C and D in the protective effects of cell-mediated immunity for fetal death may have been attributable to other undocumented causes of fetal death, sampling differences, or small sample sizes. The variation in response among farms may reflect differences in the inherent properties of farm-specific viruses. Thanawongnuwech and Thacker[26] reported that a high-virulence PRRSV isolate is associated with higher numbers of INF-γ-SC cells in the lung, compared with

a low-virulence isolate.[25] Virulent viral strains tend to replicate more efficiently in animals, compared with benign strains,[29] which may account for differences between farms in the rate and magnitude of development of the CMI response.

Overall, results of our study indicated that the association between cell-mediated immunity and protection against clinical PRRS in the field varies in herds. Nevertheless, strong trends between cell-mediated immunity and protection against clinical PRRS in some herds indicated that cell-mediated immunity may be an important predictor of clinical protection. Results of our study also indicated that a high degree of variation exists among individual sows in the intensity of the CMI response. In all 4 herds, the prevalence of antibodies against PRRSV as detected by ELISA was nearly 100% prior to sampling, suggesting that almost all sows had been exposed to PRRSV. Despite near universal exposure, several sows nevertheless had low numbers of PRRSV-specific, INF-γ-SC cells. The reasons for this variation are not clear. The number of PRRSV-specific, INF-γ-SC cells increases slowly after exposure,[20] such that the time of exposure could be 1 source of variation. Also, animals may vary inherently in their immune competence, such that some individuals within farms are innately able to mount strong CMI responses, whereas others are not.[30,31] Multiple viral genotypes have been documented on the same farm,[32,33] and these could conceivably be different in their ability to stimulate cell-mediated immunity. Additionally, despite the fact that the sows sampled from any 1 farm were housed together, differences undoubtedly existed in their individual environments, which may have had unquantified but important effects on immune competence.

Results of the study reported here suggested that if the sources of intra- and interfarm variation in the intensity of cell-mediated immunity to PRRSV can be identified, increasing levels of PRRSV-specific, cell-mediated immunity within infected herds have the potential to decrease clinical reproductive disease and increase the number of pigs weaned. Additional studies are required to understand the role of management practices, unmanaged environmental factors, innate differences among individual pigs, and viral variation in the development of PRRSV-specific immunity on farms. Development of universally successful management strategies is unlikely until the importance of these factors is known.

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a. Serum-separator Vacutainer, 7 mL, Becton, Dickinson & Co, Franklin Lakes, NJ.
b. Heparin-coated Vacutainer, 10 mL, Becton, Dickinson & Co, Franklin Lakes, NJ.
c. Herd Check PRRS ELISA, Idexx Labs, Westbrook, Me.
d. Fluorescent focus neutralization test, Veterinary Diagnostic Laboratory, South Dakota State University, Bookings, SD.
e. PROC TTEST, SAS for Windows, version 8.2, SAS Institute Inc, Cary, NC.
f. PROC MEANS, SAS for Windows, version 8.2, SAS Institute Inc, Cary, NC.
g. PROC LOGISTIC, SAS for Windows, version 8.2, SAS Institute Inc, Cary, NC.
h. PROC REG, SAS for Windows, version 8.2, SAS Institute Inc, Cary, NC.

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New Veterinary Biologic Products

Product name	Species and indications for use	Route of administration	Remarks
Scrapie Antigen Test Kit, ELISA (Bio-Rad Laboratories, Inc, US Vet Permittee No. 624)	Aids in the diagnosis of scrapie from sheep obex or retropharyngeal lymph node tissues	Not applicable	USDA permitted 03/4/05