Interaction between Porcine Reproductive-Respiratory Syndrome Virus and Bacterial Endotoxin in the Lungs of Pigs: Potentiation of Cytokine Production and Respiratory Disease

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Porcine reproductive-respiratory syndrome virus (PRRSV) is a key agent in multifactorial respiratory disease of swine. Intratracheal administration of bacterial lipopolysaccharides (LPSs) to PRRSV-infected pigs results in markedly enhanced respiratory disease, whereas the inoculation of each component alone results in largely subclinical disease. This study examines whether PRRSV-LPS-induced respiratory disease is associated with the excessive production of proinflammatory cytokines in the lungs. Gnotobiotic pigs were inoculated intratracheally with PRRSV and then with LPS at 3, 5, 7, 10, or 14 days of infection and euthanatized 6 h after LPS inoculation. Controls were inoculated with PRRSV or LPS only or with phosphate-buffered saline. Virus titers, (histo)pathological changes in the lungs, numbers of inflammatory cells, and bioactive tumor necrosis factor alpha (TNF- α), interleukin-1 (IL-1), and IL-6 levels in bronchoalveolar lavage fluids were examined. All pigs inoculated with PRRSV-LPS developed severe respiratory disease, whereas the controls that were inoculated with PRRSV or LPS alone did not. PRRSV infection significantly enhanced cytokine production in response to LPS. Peak TNF- α , IL-1, and IL-6 titers were 10 to 100 times higher in the PRRSV-LPS-inoculated pigs than in the pigs inoculated with PRRSV or LPS alone; and the titers correlated with the respiratory signs. The levels of neutrophil infiltration and the pathological changes detected in the lungs of PRRSV-LPS-inoculated pigs resembled those detected when the effects of PRRSV and LPS inoculated alone are combined, but with no synergistic effects between PRRSV and LPS. These data demonstrate a synergism between PRRSV and LPS in the induction of proinflammatory cytokines and an association between induction of these cytokines and disease.

European strains of porcine reproductive-respiratory syndrome virus (PRRSV) fail to cause respiratory disease as such. Nevertheless, PRRSV is considered one of the most important etiological agents in multifactorial respiratory disease of swine both in Europe and in the United States (18). Few studies, however, have been able to reproduce clinical respiratory disease by experimental inoculation of PRRSV followed by inoculation of a secondary virus or bacterium (4, 6, 17, 21). Variations in the severities of clinical signs and a lack of reproducibility are the main problems with these types of studies. Even a single experimental infection with respiratory viruses results in intrinsic variations in virological, inflammatory, and clinical parameters. Therefore, a second infection may enhance this variation, as the outcome of the second infection is in part dependent on that of the first infection.

We have previously developed an alternative dual-inoculation model consisting of a primary inoculation with PRRSV followed by inoculation of a nonreplicating agent, namely, lipopolysaccharide (LPS) from *Escherichia coli* (8). LPS is a major component of the outer membrane and the main endotoxin of gram-negative bacteria. Intratracheal administration of LPS (20 μ g/kg of body weight) to PRRSV-infected pigs resulted in severe respiratory disease, characterized by tachypnea, abdominal breathing, dyspnea, and high fever. In con-

trast, inoculation of PRRSV or LPS alone resulted in subclinical or mild disease. This model proved to be reproducible, in contrast to the classic dual-infection models consisting of inoculation of PRRSV followed by inoculation of a second replicating agent. In addition, we believe that the combination of PRRSV and LPS has practical relevance. Most pigs become infected with PRRSV between 4 and 16 weeks of age, and the virus persists in the lungs for up to 40 days after inoculation (9; B. Mateusen, D. Maes, H. Nauwynck, B. Balis, M. Verdonck, and A. de Kruif, Proc. 17th IPVS Congr., vol. 2, p. 240, 2002). Also, most pigs are exposed to LPS under farm conditions, as LPS is present in stable dust at concentrations ranging up to 4.9 μ g/m³. Furthermore, LPS is released at high concentrations in the lungs during pulmonary infections with gram-negative bacteria (16, 27).

The proinflammatory cytokines interleukin-1 (IL-1), tumor necrosis factor- α (TNF- α), and IL-6 are important mediators of several respiratory diseases. IL-1 and TNF- α are among the first cytokines that are produced in the lungs during an infection. They cause infiltration and activation of leukocytes in the lungs, increased microvascular permeability, and pulmonary dysfunctions (3, 20, 25). IL-1 and TNF- α also induce a cascade of secondary cytokines, such as IL-6. IL-6 is a potent inducer of acute-phase proteins in the liver (12). Although IL-6 is generally considered a proinflammatory cytokine, it also has some anti-inflammatory properties (19). IL-6 can down-regulate the production of IL-1 and TNF- α and suppress their activities by inducing the production of IL-1 receptor antagonists and soluble TNF- α receptors. Furthermore, the production of each of

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TABLE 1. Respiratory scores, virus titers, and numbers of inflammatory cells in BAL fluids

Inoculum	No. of pigs	Time of euthanasia after inoculation with:		Mean respiratory score ^a	Mean virus titer (log ₁₀ TCID ₅₀ ^b /g)	Mean no. (10 ⁶) of BAL cells	
		PRRSV (days)	LPS (h)			Neutrophils	Mononuclear cells
PBS	5	— ^c	—	0	Negative	2 ± 2	115 ± 52
PRRSV	3	3	—	0	4.4 ± 1.1	5 ± 8	154 ± 115
	3	5	—	0	5.4 ± 1.3	4 ± 3	117 ± 42
	3	7	—	0	6.0 ± 0.6	7 ± 2	216 ± 71
	4	10	—	0	5.7 ± 0.9	38 ± 38	409 ± 219
	1	14	—	0	6.0	3	337
LPS	5	—	6	0	Negative	303 ± 105	233 ± 60
PRRSV-LPS	2	3	6	2 ± 1.4	6.0 ± 0	18 ± 23	121 ± 22
	3	5	6	3 ± 0	5.1 ± 2.2	296 ± 163	208 ± 14
	6	7	6	3.2 ± 0.8	5.5 ± 0.3	320 ± 263	275 ± 144
	2	10	6	2.5 ± 0.7	5.9 ± 1.3	380 ± 474	486 ± 142
	1	14	6	3	5.7	576	483

^a Respiratory scores range from 0 to 4 (see text for the definition of each score).

^b TCID₅₀, 50% tissue culture infective dose.

^c —, not applicable.

the three cytokines in the lungs has been associated with general signs of disease such as fever, depression, and anorexia.

The present study was undertaken to test the hypothesis that PRRSV-LPS-induced respiratory disease is associated with the excessive production of proinflammatory cytokines in the lungs. Therefore, we compared the levels of production of IL-1, TNF- α , and IL-6 in the lungs of pigs after dual inoculation of PRRSV and LPS with those after the inoculation of each agent alone. Correlations between cytokine levels and respiratory signs, macroscopic and microscopic lung pathologies, and the infiltration of inflammatory cells in the bronchoalveolar spaces were examined.

MATERIALS AND METHODS

Virus and LPS preparations. PRRSV (Lelystad virus strain) (26) was used in the present study. The virus used for inoculation was at the fifth passage in alveolar macrophages, which had been obtained from 4- to 6-week-old gnotobiotic pigs. The inoculation dose was 10⁶ 50% tissue culture infective doses per pig. *E. coli* O111:B4 LPS was obtained from Difco Laboratories (Detroit, Mich.) and was used at a dose of 20 μ g/kg of body weight. This dose was based on data from earlier experiments and was selected because it caused no clinical disease and minimal IL-1 and TNF- α secretion into the lungs (24). Virus and LPS were diluted in sterile pyrogen-free phosphate-buffered saline (PBS; Gibco, Merelbeke, Belgium) to obtain a 3-ml inoculum.

Pigs, experimental design, and sampling. Thirty-eight colostrum-deprived pigs (age, 4 weeks) delivered by cesarean section were used in the study. They were housed in individual Horsefall-type isolation units with positive-pressure ventilation and were fed commercial ultrahigh-temperature-treated cow's milk. All inoculations were performed intratracheally with an 18-gauge needle that was inserted through the skin cranial to the sternum.

The pigs were allocated to four groups (see Table 1). Fourteen pigs were inoculated with PRRSV; and 3 ($n = 2$), 5 ($n = 3$), 7 ($n = 6$), 10 ($n = 2$), or 14 ($n = 1$) days later they were inoculated with LPS (PRRSV-LPS group). These pigs were euthanized 6 h after the LPS inoculation. This time point was chosen because previous virus-LPS experiments showed that cytokine production peaks at 3 to 8 h after the LPS inoculation and declines afterwards (24). Fourteen pigs were inoculated exclusively with PRRSV and euthanized at 3 ($n = 3$), 5 ($n = 3$), 7 ($n = 3$), 10 ($n = 4$), and 14 ($n = 1$) days after inoculation (PRRSV control group). Five pigs were inoculated exclusively with LPS and euthanized 6 h later (LPS control group). Five pigs were mock inoculated with PBS and euthanized 6 h later (PBS control group). All pigs were clinically monitored until euthanasia.

Samples from the left lung were collected for virological, histopathological, and standard bacteriological examinations. The right lung was used for lung

lavage by a method described earlier (22). The bronchoalveolar lavage (BAL) fluids that were recovered were separated into cells and cell-free fluids by centrifugation (400 $\times$ g, 10 min, 4°C). For four of the six pigs that were inoculated with PRRSV and then with LPS 7 days later, both the left and right lungs were used for lung lavage.

Clinical and pathological examinations. Pigs were monitored for clinical signs daily throughout the experiment and every hour after the LPS inoculation. A respiratory disease score was attributed to each pig at the time of euthanasia. Scores ranged from 0 to 4 (0, normal; 1, tachypnea when stressed; 2, tachypnea at rest; 3, tachypnea and dyspnea at rest; 4, severe tachypnea and dyspnea with labored, jerky breathing).

Macroscopic lung lesions were evaluated by visual inspection. For histopathological examination, samples of the cardiac and diaphragmatic lung lobes were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin.

BAL fluid cells were counted in a Türk chamber, and cytocentrifuge preparations were stained with Diff-Quik (Baxter, Düringen, Switzerland) to determine the percentage of neutrophils and mononuclear cells.

Cytokine bioassays. Cell-free BAL fluids were concentrated 20 times by dialysis against a 20% (wt/vol) solution of polyethylene glycol (molecular weight, 20,000) and cleared of residual virus by centrifugation at 100,000 $\times$ g before analysis in bioassays for cytokines. Bioassays for IL-1, IL-6, and TNF- α have been described in detail elsewhere (7, 23).

IL-1 was assayed by determination of its capacity to stimulate proliferation of D10(N4)M cells in the presence of concanavalin A (grade IV; Sigma, Bornem, Belgium) and recombinant human IL-2 (Genzyme, Cambridge, Mass.). The percentage of proliferation was determined by the thiazolyl blue [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] conversion procedure, and optical densities were measured. The number of biological units per milliliter of BAL fluid was determined as the dilution that produced 50% maximal proliferation. To confirm the specificity of the bioassay, D10 cells were incubated with monoclonal rat anti-mouse IL-1 receptor type 1 antibodies (Genzyme).

TNF- α activity was measured in a cytotoxicity assay with porcine kidney (PK) (15) subclone 15 cells (a gift from G. Bertoni, Bern, Switzerland) in the presence of actinomycin D. The plates were stained with crystal violet and read spectrophotometrically. The number of biological units per milliliter was defined as the dilution that produced 50% cytotoxicity. Specificity was demonstrated by neutralization of samples with rabbit anti-human TNF- α antibodies (Innogenetics, Zwijnaarde, Belgium).

IL-6 was assayed by determination of its capacity to stimulate proliferation of B9 cells (a gift from L. A. Aarden, Amsterdam, The Netherlands). Percent proliferation was determined by the thiazolyl blue [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] conversion procedure, and optical densities were measured. The number of biological units per milliliter of BAL fluid was determined as the dilution that produced 50% maximal proliferation. To confirm

FIG. 1. Hematoxylin-eosin staining of the lungs of pigs inoculated with PBS only (a), LPS only (b), PRRSV only (10 days after inoculation) (c), and PRRSV and then LPS over a 10-day interval (d). Inter-alveolar septal thickening was comparable in pigs inoculated with PRRSV-LPS and pigs inoculated with PRRSV alone. Magnifications, $\times 100$.

the specificity of the bioassay, samples were neutralized with goat anti-porcine IL-6 antibodies (R&D Systems, Abingdon, United Kingdom).

Bioassays were done with twofold dilutions of samples in 96-well microtitration plates. Laboratory standards were run in each bioassay. Samples were tested in two or three individual bioassays, and the geometric means were calculated.

Virological and bacteriological examinations. Tissue samples from the diaphragmatic lobe of the left lung were used for virological and bacteriological examinations. PRRSV titrations were performed on alveolar macrophages by standard methods (26). For bacteriology, samples of lung tissue were plated on bovine blood agar and cultured aerobically. A nurse colony of coagulase-positive *Staphylococcus* species was streaked diagonally on each plate. The plates were inspected for bacterial growth after 48 and 72 h. The colonies were then identified by standard techniques.

Statistical analysis. Standard two-sample Mann-Whitney tests were used to compare respiratory disease scores and cytokine titers. Correlation coefficients were calculated by the Spearman rank correlation test. *P* values < 0.05 were considered significant. Statistical analyses were performed by using SPSS (version 6.1) software.

RESULTS

The lungs of all pigs were free of bacteria by culture. PRRSV titers are presented in Table 1. PRRSV was isolated from the lungs of all virus-inoculated pigs but not from pigs inoculated with LPS or PBS only. There were no differences in virus titers between the pigs inoculated with PRRSV and LPS

combined and the pigs inoculated with PRRSV alone or at the different time points after inoculation of PRRSV.

Clinical signs. Mean respiratory scores are presented in Table 1. Pigs that received PBS or LPS only remained asymptomatic. Pigs inoculated with PRRSV only showed no respiratory signs at any day after inoculation. They showed mild anorexia and dullness between 3 and 5 days after inoculation.

In contrast, all pigs inoculated with PRRSV-LPS developed marked respiratory signs. All pigs were clinically normal before the LPS inoculation but developed tachypnea, dyspnea with labored, abdominal breathing, and depression within 1 to 2 h after LPS. These signs were still present at the time of euthanasia. There were no differences in disease severity among the pigs inoculated with LPS at 3, 5, 7, 10, or 14 days after inoculation of PRRSV. Respiratory disease scores were significantly ($P < 0.05$) higher for the group inoculated with PRRSV-LPS than for any other group.

Macroscopic and microscopic lung pathologies. PBS-treated control pigs did not have macroscopic or microscopic lung pathologies (Fig. 1). The lungs of PRRSV-inoculated pigs had a mottled appearance with multifocal red and tan areas. Multifocal interstitial pneumonia was found microscopically. Inter-

alveolar septal thickening with infiltration of mononuclear cells was the major feature and increased from 3 to 14 days after PRRSV inoculation. Inoculation with LPS only resulted in milder pneumonic lesions. Macroscopic lesions were characterized by focal areas of atelectasis and interlobular edema. The characteristic histopathological features were thickening of the interalveolar septa, although it was less pronounced than that after inoculation with PRRSV, and bronchiolar infiltration with neutrophils and macrophages. Intra-alveolar edema and focal transudation of erythrocytes were occasionally seen.

The macroscopic and microscopic lung lesions after PRRSV-LPS inoculation resembled the combination of the lesions seen after inoculation of PRRSV or LPS alone. The lungs were mottled with small red and tan areas and interlobular edema. Microscopically, there was thickening of the interalveolar septa due to an infiltration of mononuclear cells and neutrophils. The degree of septal thickening was comparable to that seen after inoculation of PRRSV only.

Infiltration of inflammatory cells. The BAL cells of the PBS-treated control pigs consisted mainly of mononuclear cells (mean, 115×10^6 cells) and a few neutrophils (mean, 2×10^6 cells) (Table 1). PRRSV-inoculated pigs showed an influx of mononuclear cells in the bronchoalveolar spaces, and this influx increased from 3 to 14 days after inoculation. Starting at 7 days after inoculation with PRRSV, the mean number of mononuclear cells was at least two times higher in PRRSV-inoculated pigs than in PBS-treated control pigs. The number of neutrophils in all except one of the PRRSV-inoculated pigs was comparable to that in PBS-treated control pigs; one PRRSV-inoculated pig had 91×10^6 neutrophils. The LPS inoculation induced infiltration of both neutrophils (mean, 303×10^6 cells) and mononuclear cells (mean, 233×10^6 cells).

Pigs inoculated with PRRSV-LPS showed an influx of both mononuclear cells and neutrophils. The amount and kinetics of mononuclear cell infiltration in the pigs inoculated with PRRSV-LPS were comparable to those in the PRRSV-inoculated control pigs. The neutrophil numbers in the pigs inoculated with PRRSV-LPS, on the other hand, were generally comparable to those in the LPS-inoculated control pigs. Only 3 of the 14 pigs inoculated with PRRSV-LPS had larger numbers of neutrophils (567×10^6 to 786×10^6) than the LPS-inoculated control pigs. One pig inoculated with PRRSV-LPS showed no neutrophil infiltration at all (1×10^6), and three pigs inoculated with PRRSV-LPS showed only minor neutrophil infiltration (19×10^6 to 44×10^6) compared to that detected in the LPS-inoculated control pigs. Two of these pigs were inoculated with LPS 3 days after PRRSV inoculation, which explains the low mean number of neutrophils in this group.

Biologically active IL-1, TNF- α , and IL-6 in BAL fluids.

Figure 2 shows the IL-1, TNF- α , and IL-6 titers in the BAL fluids of individual pigs after inoculation of PRRSV-LPS, PRRSV only, and LPS only. PBS-treated control pigs had no detectable IL-1, TNF- α , or IL-6. Ten of 14 PRRSV-inoculated pigs had elevated IL-1 titers, with the highest titers (183 to 339 U/ml) detected 10 days after inoculation. Only 3 of these 14 pigs (which were euthanatized 7, 10, and 14 days after inoculation, respectively) had detectable TNF- α titers (28 to 61 U/ml). Ten pigs had detectable IL-6 titers (61 to 343 U/ml). LPS inoculation induced the production of all three cytokines

FIG. 2. Titers of proinflammatory cytokines in BAL fluids of PRRSV-LPS-inoculated pigs and pigs inoculated with PRRSV only or LPS only. Each dot corresponds to one pig: ●, pigs inoculated with LPS at the indicated day after PRRSV inoculation; ○, pigs inoculated with PRRSV only; △, pigs inoculated with LPS only. The dotted line represents the detection limit.

in the lungs. IL-1 (titers, 28 to $1,022$ U/ml) and IL-6 (titers, $1,276$ to $2,659$ U/ml) were detected in all five pigs, and TNF- α (titers, 28 to 133 U/ml) was detected in three pigs.

Compared to the pigs inoculated with PRRSV and LPS alone, 10 of 14 pigs inoculated with PRRSV-LPS showed significantly ($P < 0.05$) increased titers of at least one of the three cytokines. In nine pigs the titers of IL-1 (titers, $2,172$ to $20,480$ U/ml), TNF- α (titers, 164 to $6,047$ U/ml), and IL-6 (titers, $2,511$ to $378,724$ U/ml) were strongly increased; and in one pig only the titer of IL-1 ($2,840$ U/ml) was increased. The highest cytokine titers were detected in pigs inoculated with LPS 5 to 14 days after the PRRSV inoculation, and they were 10 to 100 times higher than the cytokine titers of the control pigs inoculated with PRRSV or LPS only. Four pigs inoculated with

TABLE 2. Correlation coefficients between respiratory scores, cytokine titers, and numbers of inflammatory cells in BAL fluids

Parameter	Correlation with:					
	Respiratory score	IL-1 titer	TNF- α titer	IL-6 titer	Neutrophils no.	Mononuclear cell no.
Respiratory score	11	0.81	0.70	0.71	0.59	NS ^a
IL-1 titer	— ^b	1	0.75	0.85	0.80	0.43
TNF- α titer	—	—	1	0.84	0.74	0.40
IL-6 titer	—	—	—	1	0.84	NS
Neutrophil no.	—	—	—	—	1	0.61
Mononuclear cell no.	—	—	—	—	—	1

^a NS, no significant correlation ($P > 0.05$).

^b —, not applicable.

PRRSV-LPS, on the other hand, did not show enhanced levels of cytokine production. These pigs had negligible levels of TNF- α (titers, <20 to 31 U/ml), and the levels of IL-1 (titers, 191 to 1,571 U/ml) and IL-6 (titers, 266 to 2425 U/ml) were comparable to those for the LPS-treated control pigs.

The left and right lungs of pigs that were inoculated with PRRSV-LPS and whose lungs were lavaged showed no difference in cytokine titers or cell counts ($P > 0.05$) (data not shown).

Table 2 presents the correlation between respiratory scores, cytokine levels, and numbers of inflammatory cells in BAL fluids. The levels of all three cytokines were tightly correlated with each other and with the respiratory scores and the neutrophil numbers. There was, however, little correlation between neutrophil numbers and respiratory scores. The number of infiltrated mononuclear cells did not correlate with cytokine levels or respiratory scores. The four pigs inoculated with PRRSV-LPS that did not have increases in cytokine levels also had lower neutrophil numbers (1×10^6 to 129×10^6). The cytokine titers and BAL cell numbers did not correlate with the virus titers (data not shown).

DISCUSSION

This study demonstrates that a PRRSV infection sensitizes the lungs for production of proinflammatory cytokines upon exposure to LPS. Moreover, the cytokine titers were tightly correlated with the appearance of respiratory signs. We have previously documented a similar phenomenon for another respiratory virus of swine that causes subclinical disease, porcine respiratory coronavirus (PRCV) (24). Like PRRSV, PRCV infection enhanced the levels of production of TNF- α and IL-1 in response to LPS, and the levels of both cytokines correlated with the severity of disease. The pathogenesis of PRRSV-LPS-induced disease appears to be similar to the pathogenesis of PRCV-LPS-induced disease. As IL-1 and TNF- α have overlapping effects and potentiate the effects of each other, we consider them both to be central mediators in virus-LPS-induced disease. IL-6 levels in the lungs of pigs inoculated with both virus and LPS were assessed for the first time in the present study, and they were also found to be markedly enhanced. IL-6 is probably induced as a secondary cytokine in response to IL-1 and TNF- α , which may explain the tight correlation between IL-6 levels and IL-1 and TNF- α levels. Because IL-6 has both pro- and anti-inflammatory activities, it may either contribute to disease or counteract the activities of IL-1 and TNF- α .

We have indications that the tachypnea and dyspnea resulting from PRRSV-LPS or PRCV-LPS inoculations are due to a functional process, such as bronchoconstriction, rather than to structural lung damage. First, the onset of respiratory signs is hyperacute. In another study of PRRSV-LPS inoculation, it was shown that respiratory signs started within 1 h after LPS inoculation, reached a climax 2 to 4 h later, and were clearly diminished 12 h later (8). Second, the microscopic lesions in the lungs of pigs inoculated with PRRSV-LPS and with PRRSV only did not differ much. Pigs in both groups had interstitial pneumonia typical of PRRSV infection, and LPS inoculation had little extra effect. The inoculation with LPS as such caused a marked increase in the numbers of neutrophils in BAL fluids, but there were no differences in neutrophil numbers between pigs inoculated with PRRSV-LPS and those inoculated with LPS alone. Third, it is well known that IL-1 and TNF- α can cause bronchial hyperreactivity (2, 15) and bronchoconstriction (10), leading to asthma-like symptoms. Moreover, TNF- α and IL-1 were shown to act synergistically in the induction of bronchoconstriction in the rat lung (10). Therefore, simultaneous overproduction of these cytokines after PRRSV-LPS inoculation may cause increased and sustained contraction of bronchi, which may explain the acute respiratory signs.

We cannot explain why four pigs inoculated with PRRSV-LPS, which showed clear respiratory signs, had only low cytokine titers and negligible neutrophil infiltration. There were no consistent differences in PRRSV titers or the numbers of mononuclear cells in BAL fluids between these and the other pigs. Because LPS exerts its effect locally, our initial hypothesis was that the LPS inoculum probably did not reach the right lung in those pigs and that cytokine production and neutrophil infiltration might have been restricted to the left lung. To test this hypothesis we lavaged both the left and the right lungs of four pigs inoculated with PRRSV-LPS. There were no differences in the levels of cytokine production or neutrophil infiltration between the two lung halves. Therefore, it can be assumed that the LPS inoculum is distributed equally between both lung halves in most pigs. The true reason for the variability in cytokine production and neutrophil infiltration among pigs inoculated with PRRSV-LPS is unclear.

There have been few studies on the interactions between viruses and LPS in vivo. To our knowledge, PRRSV and PRCV are the first respiratory viruses shown to act synergistically with LPS in the induction of respiratory disease and cytokines. Recently, it has been described that systemic infec-

tion of mice with lymphocytic choriomeningitis virus or vesicular stomatitis virus leads to fatal shock upon intraperitoneal inoculation of a sublethal dose of LPS (13, 14). It appeared that the shock syndrome was caused by the overproduction of TNF- α . Mice inoculated with virus-LPS had 3- to 50-fold higher serum TNF- α levels compared to those in the sera of mice inoculated with LPS alone. By use of knockout mice, it was demonstrated that virus-induced interferon was responsible for the increased sensitivity to LPS (5, 13). Both alpha/beta interferon and gamma interferon were able to sensitize cells to LPS. It is unlikely, however, that alpha interferon is involved in the sensitization of PRRSV-infected pigs to LPS, because alpha interferon production is minimal during infection with PRRSV (1, 23).

It remains to be seen whether the PRRSV-induced infiltration of the lungs with mononuclear cells contributes to the increased responsiveness to LPS. PRRSV induces infiltration of monocytes in the lungs, reaching a peak at 25 days after inoculation (9). In mice, it was shown that monocytes infiltrating the lungs in response to monocyte chemoattractant protein type 1 (MCP-1) have increased levels of expression of CD14, the LPS receptor, and become primed for enhanced TNF- α production in response to LPS (11). It is possible that monocytes attracted to PRRSV are an important source of cytokines upon LPS exposure and that they are responsible for the enhanced cytokine response compared to the response of uninfected lungs. In this study, the number of mononuclear cells in the bronchoalveolar spaces did not correlate with the respiratory signs. There are two important considerations in this regard. First, the profiles of the cells in the BAL fluid of pigs inoculated with PRRSV-LPS were partly the result of the LPS inoculation and, as such, did not reflect the situation before the LPS inoculation. Second, we counted the mononuclear cells in the BAL fluids and not in the interstitium, while interstitial monocytes may be important targets for LPS.

In conclusion, respiratory viruses like PRRSV, which do not cause respiratory signs on their own, can sensitize the lungs for the production of proinflammatory cytokines and respiratory signs upon exposure to bacterial endotoxins. This interaction may be important in the development of multifactorial respiratory disease, as is often seen in the field.

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