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Abstract

The objective of this study was to determine whether host genetics play a role in susceptibility to the respiratory disease in growing pigs caused by the porcine reproductive and respiratory syndrome virus (PRRSV). Based on a previous study, 2 genetically diverse commercial lines of pigs that also were divergent in the susceptibility of monocyte-derived macrophages to PRRSV infection in vitro were selected for an in vivo challenge study. Based on the average percentage of infected macrophages for each line, a line derived from the Large White breed was characterized as fluorescence-activated cell sorting^{hi} (FACS^{hi}), and a line derived from Duroc and Pietrain breeds was characterized as FACS^{lo}. Pigs from each line were challenged at 6 wk of age with PRRSV VR-2385 and necropsied at 10 or 21 d after infection. Data collected included clinical evaluation of disease, virus titration in serum and lung lavage fluid, macroscopic lung lesion scores, and microscopic lung lesion scores. The FACS^{lo} line had consistently more severe clinical disease compared with the FACS^{hi} line in the early stages of infection. Differences between line means were significant ($P < 0.05$) at 10 d after infection for all variables just described, and the FACS^{lo} line showed more severe signs of disease. By 21 d after infection, clinical signs and lesions were resolving, and the differences between lines were significant ($P < 0.04$) only for microscopic lung lesion scores but approached significance ($P < 0.08$) for virus titer in serum. At 21 d after infection, the relationship between the lines reversed; the FACS^{hi} line had higher serum virus titers than the FACS^{lo} line. This report provides evidence that strongly suggests the existence of a host genetic component in disease susceptibility to PRRSV and indicates that further study is warranted to define the cellular mechanisms that affect disease susceptibility.

Keywords

genetic susceptibility, pig, porcine reproductive and respiratory syndrome virus, respiratory disease

Disciplines

Agriculture | Animal Sciences | Genetics and Genomics | Veterinary Medicine

Comments

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An investigation of susceptibility to porcine reproductive and respiratory syndrome virus between two genetically diverse commercial lines of pigs[1]

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ABSTRACT: The objective of this study was to determine whether host genetics play a role in susceptibility to the respiratory disease in growing pigs caused by the porcine reproductive and respiratory syndrome virus (PRRSV). Based on a previous study, 2 genetically diverse commercial lines of pigs that also were divergent in the susceptibility of monocyte-derived macrophages to PRRSV infection in vitro were selected for an in vivo challenge study. Based on the average percentage of infected macrophages for each line, a line derived from the Large White breed was characterized as fluorescence-activated cell sorting^{hi} (FACS^{hi}), and a line derived from Duroc and Pietrain breeds was characterized as FACS^{lo}. Pigs from each line were challenged at 6 wk of age with PRRSV VR-2385 and necropsied at 10 or 21 d after infection. Data collected included clinical evaluation of disease, virus titration in serum and lung lavage fluid, macroscopic lung lesion scores, and microscopic lung lesion scores. The FACS^{lo} line had consistently more severe clinical disease compared with the FACS^{hi} line in the early stages of infection. Differences between line means were significant ($P < 0.05$) at 10 d after infection for all variables just described, and the FACS^{lo} line showed more severe signs of disease. By 21 d after infection, clinical signs and lesions were resolving, and the differences between lines were significant ($P < 0.04$) only for microscopic lung lesion scores but approached significance ($P < 0.08$) for virus titer in serum. At 21 d after infection, the relationship between the lines reversed; the FACS^{hi} line had higher serum virus titers than the FACS^{lo} line. This report provides evidence that strongly suggests the existence of a host genetic component in disease susceptibility to PRRSV and indicates that further study is warranted to define the cellular mechanisms that affect disease susceptibility.

Key words: genetic susceptibility, pig, porcine reproductive and respiratory syndrome virus, respiratory disease

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INTRODUCTION

Porcine reproductive and respiratory syndrome virus (**PRRSV**) is an enveloped RNA virus in the family *Arteriviridae* that is responsible for considerable financial losses caused by respiratory and reproductive disease in pigs (Benfield et al., 1992). In growing pigs, PRRSV causes an interstitial pneumonia (Collins et al., 1992) resulting in fever, lethargy, and respiratory distress. Respiratory disease has been experimentally reproduced through intranasal challenge with high-virulence, low-passage PRRSV (Halbur et al., 1995). The PRRSV preferentially infects resident macrophages of various tissues, including pulmonary alveolar macrophages. Varying levels of disease induced by a single strain of PRRSV between pigs of the same population have been observed in naturally and experimentally infected animals (Halbur et al., 1998) as well as in macrophages infected in vitro (Voicu et al., 1994; Molitor et al., 1996). Halbur et al. (1998) investigated differences between purebred pigs, but reports of studies with commercial crossbred lines were not found. If evidence of genetic control of susceptibility to PRRSV could be identified, the potential for selection of pigs that have increased resistance to this important disease could be

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considered (Stear et al., 2001; van der Waaij et al., 2002).

Primary cultures of pulmonary alveolar macrophages and monocyte-derived macrophages (**MDM**) can be infected in vitro; positive cells were detected through fluorescence-activated cell sorting (**FACS**; Thacker et al., 1998). A FACS assay that detects the percentage of PRRSV-infected MDM in vitro has been used to demonstrate the presence of variation between genetic lines of pigs based on the average percentage of infected MDM (Vincent et al., 2005); however, association with measures of disease in vivo was not assessed. Our objective was to investigate differences in vivo between the $FACS^{hi}$ and $FACS^{lo}$ lines based on clinical presentation, macroscopic and microscopic lesions, and virus titers in serum.

MATERIALS AND METHODS

Animals

Two hundred sixty-four animals, 2 to 4 wk of age, from the $FACS^{hi}$ (Large White-based) and $FACS^{lo}$ (Duroc and Pietrain-based) lines were procured from the same PRRSV-free source farm in 4 independent replicates. The group representing the $FACS^{hi}$ line included offspring from 11 sires and 29 litters. The group representing the $FACS^{lo}$ line included offspring from 11 sires and 33 litters. Each pig was treated daily by i.m. injection with lincomycin hydrochloride (Lincocin, Pharmacia & Upjohn, Kalamazoo, MI) and ceftiofur hydrochloride (Excenel, Pharmacia & Upjohn) for 3 d after arrival. The initial replicate contained 30 animals per line in the challenge group, and pigs with the same sire were sorted randomly into 6 pens of 10 pigs each. The later three replicates contained 24 animals per line in the challenge group, and pigs with the same sire were sorted randomly into 6 pens of 8 pigs each. Additional animals (n = 60) used as experimental negative controls were housed in a separate isolation facility during each respective replicate. Pigs were infected intranasally at approximately 6 wk of age (0 d after infection) and subsequently necropsied on d 10 or 21 after infection. The study was conducted in accordance with the guidelines of the Iowa State University Institutional Committee on Animal Care and Use.

In Vitro Susceptibility of MDM

The PRRSV-MDM FACS results reported previously were analyzed with the data generated in this study to investigate the relationship between the in vitro susceptibility to PRRSV and the in vivo disease variables described here; the in vitro assay results and in vivo measurements were collected from the same groups of animals. Briefly, in each of the replicates, blood was collected from 4- to 6-wk-old pigs by venipuncture into heparinized tubes and processed the same day. Peripheral blood mononuclear cells (**PBMC**) were isolated from the blood, and the adherent monocytes were transformed into macrophages using an established protocol (Genovesi et al., 1989; Thacker et al., 1998). After 24 h, PRRSV (VR-2385) was added to the media at a multiplicity of infection of one and was allowed to incubate for another 24 h. Negative controls containing noninfected MDM were included for each pig to standardize the FACS analysis. The MDM were labeled using an antibody (74-22-15A, IgG2b isotype, VMRD, Pullman, WA) that recognizes monocytes and macrophages followed with a goat anti-mouse IgG (whole molecule) conjugated with R-phycoerythrin (Southern Biotechnology Associates, Inc., Birmingham, AL). The cells were fixed with 4% formaldehyde and then permeabilized with FACS buffer plus 0.1% saponin (FACS-saponin buffer). The intracellular nucleocapsid protein of PRRSV was labeled with the monoclonal antibody SDOW-17 (IgG1 isotype, Rural Technologies, Brookings, SD) followed with a goat anti-mouse IgG1 conjugated with fluorescein isothiocyanate (**FITC**; Southern Biotechnology Associates) in FACS-saponin buffer. The percentage of R-phycoerythrin-positive MDM that were dual-labeled with FITC, representing the PRRSV-infected macrophage population, was identified by flow cytometry based on 10,000 events.

Blood Collection and Serology

Blood was collected from all pigs before and after challenge to monitor for the presence of PRRSV antibodies. Serum samples were tested using a commercially available ELISA (HerdChek: PRRS, IDEXX Laboratories, Westbrook, ME). Samples also were collected from all pigs at 10 d after infection and from the remaining pigs at 21 d after infection for use in virus titration and molecular quantification.

Virus Inoculum and Challenge

The PRRSV inoculum was prepared as previously described (Halbur et al., 1995, 1998). A high-virulence, low-passage strain was used (VR-2385) at a dose of 10^5 to 10^6 tissue culture infective dose 50 (**$TCID_{50}$**)/mL. The inoculum was administered by dripping 2.5 mL into each nostril of the animals in each of the challenge groups. Animals were challenged at approximately 6 wk of age, and this day was designated as 0 d after infection.

Clinical Evaluation

Animals were observed daily beginning on 0 d after infection through 10 d after infection for clinical signs of disease. Rectal temperatures were measured, and pigs were evaluated and assigned a score (scale of 0 to 6) for respiratory distress using a previously described method (Halbur et al., 1996), where 0 = normal, 1 = mild dyspnea and/or tachypnea when stressed, 2 = mild dyspnea and/or tachypnea when at rest, 3 = moderate

dyspnea and/or tachypnea when stressed, 4 = moderate dyspnea and/or tachypnea when at rest, 5 = severe dyspnea and/or tachypnea when stressed, and 6 = severe dyspnea and/or tachypnea when at rest. From 10 d after infection until the final necropsy at 21 d after infection, animals were evaluated every other day. Temperatures were converted to a temperature score to indicate the number of days the animal was febrile by giving those pigs with temperatures within the 97.5 percentile of prechallenge temperatures a score of 0 (<40°C). Those with temperatures above the 97.5 percentile were considered febrile and given a score of 1 (≥40°C). The scores were totaled for each time period, 0 to 10 d, 12 to 20 d, or 0 to 20 d after infection. Respiratory scores also were totaled for each animal within each time period.

Pathological Examination

At 10 d after infection, approximately one-half of the pigs from each challenged line were necropsied, followed by the remaining one-half from each line at 21 d after infection. The lungs were removed and evaluated for macroscopic lesions, and samples were collected for culture. Each lung lobe was given a score to reflect the percentage affected by pneumonia as previously described by Halbur et al. (1995). The scores for the lobes were added to give a total lung score to indicate the percentage of the total lung with grossly visible lesions. To evaluate the overall health status of each animal, a bronchial swab was collected and cultured for swine respiratory pathogens, including *Bordetella bronchiseptica*, *Pasteurella multocida*, *Actinobacillus* spp., *Haemophilus* spp., and *Mycoplasma hyorhinis*. Samples were inoculated onto blood agar and streaked with a *Staphylococcus epidermidis* nurse colony for support of *A. pleuropneumoniae* and *Haemophilus* spp. Isolated bacteria were identified according to standard methods. The lungs were then lavaged with 50 mL of minimal essential media with antibiotics (100 U of penicillin G/mL, 100 μg of streptinomycin/mL, and 50 μg of gentamicin/mL), and bronchoalveolar lavage (**BAL**) fluid was collected. Tracheobronchial and middle iliac lymph nodes were removed and weighed. Tissue sections from all animals were taken from lung, lymph nodes, turbinate, brain, and heart and then fixed in 10% (vol/vol) neutral buffered formalin. Tissue sections were processed routinely, embedded in paraffin in an automated tissue processor, and stained with hematoxylin and eosin for histologic evaluation. Lung sections were blindly examined and given a score (scale of 0 to 6) based on the degree of interstitial pneumonia.

Virus Titration and Quantification with Quantitative Competitive-Reverse Transcriptase-PCR (QC-RT-PCR)

A standard aliquot of each serum or BAL sample from all 4 replicates was titrated in triplicate using a tissue culture method described previously (Kim et al., 1993; Meng et al., 1996). The presence of PRRSV was detected using an indirect immunofluorescence assay with a monoclonal antibody against the nucleocapsid protein (SDOW-17, Rural Technologies) and a secondary goat anti-mouse IgG (whole molecule) conjugated with FITC (Sigma Aldrich Corp., St. Louis, MO). The $TCID_{50}$ was calculated using the Reed-Muench accumulative method (Coligan et al., 1996).

For replicates 2, 3, and 4, viral RNA was extracted from a standard volume of 140 μL of sera or BAL using a commercial kit (QIAamp viral RNA Mini Kit, Qiagen Inc., Valencia, CA) according to the manufacturer's directions. Each RNA sample was eluted in 60 μL of the provided elutant. To analyze viral RNA levels, QC-RT-PCR were run in triplicate for each test serum and BAL sample with 3 different concentrations of the 405 nucleotide competitor as described previously (Vincent and Thacker, 2001). Before running each set of samples, the range of competitor was established by testing a random selection of samples against a range of competitor concentrations from 10^6 to 10^{10} copy numbers per microliter. The reactions were run on 2.5% agarose gels for approximately 1 h. The gels were stained with ethidium bromide, and the band intensities were analyzed using the digital imaging system GeneSnap (Spectronics, Westbury, NY) linked with the gel analysis software GeneTools. The intensity ratio of the competitor (C) to unknown target (T) multiplied by the ratio of sizes between the sample target and competitor (C/T/0.835) was plotted against competitor copy number on a double-log scale, and the unknown viral copy number was calculated as the point where the log of the ratio of competitor to unknown was zero (Piatak et al., 1993).

Statistical Analyses

Endpoint data were analyzed using REML mixed-model analysis, and daily rectal temperatures were analyzed using repeated measures with day in the model (Mixed procedure, ANOVA, SAS Inst., Inc., Cary, NC). For data generated from the experimental infection, line and trial replicate were assumed to be fixed effects, whereas sire and litter were assumed to be random effects; the disease traits were the dependent variables included in the model. A linear regression analysis was done using Statistix 7.0 (Analytical Software, Tallahassee, FL) to calculate Pearson correlations between the FACS results and experimental challenge clinical and disease trait measurements.

RESULTS

Means for the disease variables that were significantly different between the 2 lines are presented in Table 1.

Table 1. Summary of least squares means with *P*-values between the FACS^{hi} and FACS^{lo} lines over all 4 replicates[1]

Disease trait	*P*-value	FACS^{hi} line	FACS^{lo} line
PRRSV-FACS assay, %	<0.01	31.4 ± 2.0	23.1 ± 2.0
Respiratory score 1 to 10 d after infection	<0.04	15.4 ± 0.3	16.4 ± 0.3
No. of febrile days 1 to 10 d after infection	<0.02	1.7 ± 0.2	2.4 ± 0.2
No. of febrile days 1 to 20 d after infection	<0.05	2.2 ± 0.3	3.1 ± 0.3
Serum virus titer 10 d after infection, 10^x $TCID_{50}$/mL	<0.02	$10^{4.06 \pm 0.05}$	$10^{4.24 \pm 0.05}$
Macroscopic lung lesion 10 d after infection, %	<0.005	40.8 ± 2.8	53.5 ± 2.7
Interstitial pneumonia score (0 to 6 scale) 10 d after infection	<0.001	3.6 ± 0.1	4.5 ± 0.1
Serum virus titer 21 d after infection, 10^x $TCID_{50}$/mL	<0.08	$10^{1.5 \pm 0.28}$	$10^{0.80 \pm 0.28}$
Interstitial pneumonia score (0 to 6 scale) 21 d after infection	<0.04	1.5 ± 0.1	2.0 ± 0.1

[1]PRRSV = porcine reproductive and respiratory syndrome virus, FACS = fluorescence-activated cell sorting, and TCID = tissue culture infective dose.

Serology

All animals were serologically negative before challenge, and the negative control animals remained seronegative throughout, verifying that the animals were PRRSV-free when received.

FACS Assay

The difference in percentage of infected MDM between lines was significant ($P < 0.01$), as reported in Vincent et al. (2005). The FACS^{hi} line had an average of 31.4 ± 2.0% positive MDM, and the FACS^{lo} line had an average of 23.1 ± 2.0% positive MDM. The correlations between the FACS results and disease traits are reported in the individual sections.

Clinical Evaluation

Challenged animals from both lines began to show clinical signs of respiratory disease in addition to increased rectal temperatures 24 h after challenge. From 12 to 20 d after infection, temperatures and respiratory rates returned to normal. Over the 4 replicates, the least squares means for number of febrile days from 1 to 10 d after infection were 1.7 ± 0.2 for the FACS^{hi} line and 2.4 ± 0.2 for the FACS^{lo} line ($P < 0.02$) and from 1 to 20 d after infection ranged from 2.2 ± 0.3 for the FACS^{hi} line to 3.1 ± 0.3 for the FACS^{lo} line ($P < 0.05$). In addition, the line effect was significant in the repeated measures analysis of daily rectal temperatures ($P < 0.001$; Figure 1). The least squares means for respiratory score for 1 to 10 d after infection were 15.4 ± 0.3 for the FACS^{hi} line and 16.4 ± 0.3 ($P < 0.04$) for the FACS^{lo} line. The results of the in vitro FACS susceptibility assay were correlated with number of febrile days from 1 to 10 d after infection at r = –0.50 ($P < 0.001$), with number of febrile days from 1 to 20 d after infection at r = –0.43 ($P < 0.001$), and with respiratory score sum from 1 to 10 d after infection at r = –0.24 ($P < 0.02$). The remaining clinical measurements past 10 d after infection were not significantly correlated with FACS results.

Pathological Examination

At 10 d after infection, typical PRRSV macroscopic lesions were visible in all challenged animals. The lesions were described as multifocal to diffuse tan mottled areas and lungs that were firm and failed to collapse. There was visible lymph node enlargement in nearly all challenged animals. In addition, microscopic lung lesions typical of PRRSV were observed in all challenged animals. The least squares means for percentage of macroscopic pneumonia were 40.8 ± 2.8 for the FACS^{hi} line and 53.5 ± 2.7 for the FACS^{lo} line ($P < 0.005$). The microscopic interstitial pneumonia scores were consistent with the macroscopic pneumonia lesions with

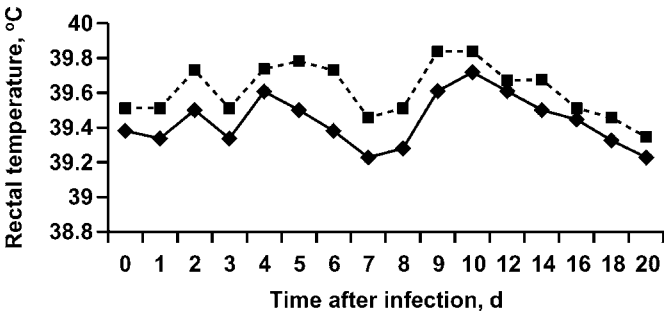

Figure 1. Daily least squares means of rectal temperatures by line. Line had a statistically significant effect over the trial period, $P < 0.001$. The fluorescence-activated cell sorting^{hi} (FACS^{hi}) line is represented by the solid line and diamond, and the FACS^{lo} line is represented by the dashed line and square.

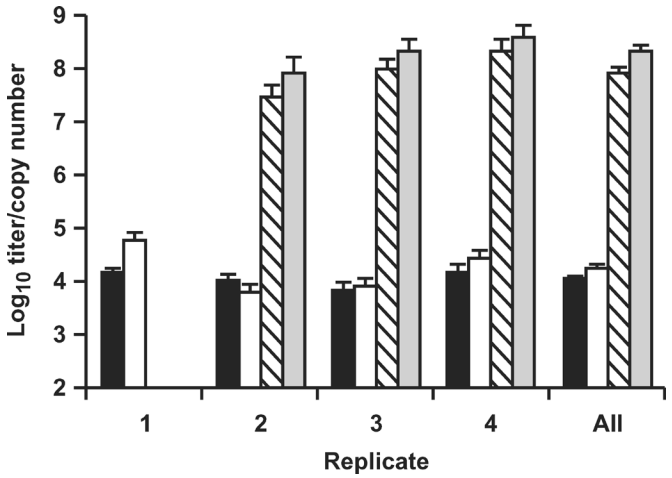

Figure 2. Log_{10}-transformed serum tissue culture titer in tissue culture infective $dose_{50}$ ($TCID_{50}$)/mL or quantitative competitive-reverse transcriptase-PCR (QC-RT-PCR) copy number per microliter at 10 d after infection. The least squares means ± SE for $TCID_{50}$/mL of serum virus titer at 10 d after infection were $10^{4.06 \pm 0.05}$ for the fluorescence-activated cell sorting^{hi} ($FACS^{hi}$) line (solid black bar) and $10^{4.24 \pm 0.05}$ for the $FACS^{lo}$ line (solid white bar) over all 4 replicates, $P < 0.02$. Least squares means for copy number per microliter in serum QC-RT-PCR results for replicates 2, 3, and 4 at 10 d after infection were $10^{7.9 \pm 0.09}$ for the $FACS^{hi}$ line (hatched bar) and $10^{8.3 \pm 0.07}$ for the $FACS^{lo}$ line (solid gray bar), $P < 0.003$.

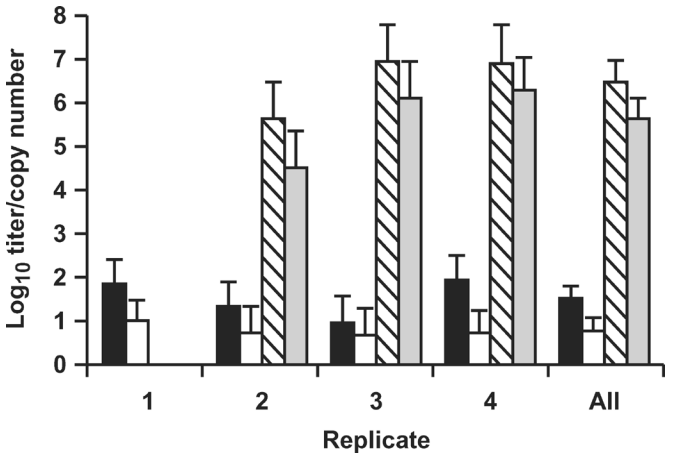

Figure 3. Log_{10}-transformed serum tissue culture titer in tissue culture infective $dose_{50}$ ($TCID_{50}$)/mL or quantitative competitive-reverse transcriptase-PCR (QC-RT-PCR) copy number per microliter at 21 d after infection. The least squares means ±SE for $TCID_{50}$/mL serum virus titer were $10^{101.54 \pm 0.28}$ for the fluorescence-activated cell sorting^{hi} ($FACS^{hi}$) line (solid black bar) and $10^{10.80 \pm 0.28}$ for the $FACS^{lo}$ line (solid white bar) over all 4 replicates, $P < 0.08$. Least squares means for copy number per microliter in serum QC-RT-PCR results for replicates 2, 3, and 4 at 21 d after infection were $10^{6.51 \pm 0.50}$ for the $FACS^{hi}$ line (hatched bar) and $10^{5.64 \pm 0.50}$ for the $FACS^{lo}$ line (solid gray bar), $P < 0.24$.

least squares means of 3.6 ± 0.1 for the $FACS^{hi}$ line and 4.5 ± 0.1 the $FACS^{lo}$ line ($P < 0.001$). The FACS results were significantly correlated with the macroscopic pneumonia scores at 10 d after infection at r = –0.28 ($P < 0.005$), but they were not correlated with microscopic interstitial pneumonia scores. The 21 d after infection, microscopic interstitial pneumonia scores had least squares means of 1.5 ± 0.1 for the $FACS^{hi}$ line and 2.0 ± 0.1 the $FACS^{lo}$ line ($P < 0.04$). There were no significant differences between lines for macroscopic lesions at 21 d after infection, and no other measurements were significantly correlated with the FACS results.

Virus Titration

All pigs that were challenged had detectable levels of virus at 10 d after infection in serum and BAL by both the tissue culture $TCID_{50}$ method and by QC-RT-PCR. The least squares means for $TCID_{50}$/mL of serum virus titer at 10 d after infection were $10^{4.06 \pm 0.05}$ for the $FACS^{hi}$ line and $10^{4.24 \pm 0.05}$ for the $FACS^{lo}$ line over all 4 replicates ($P < 0.02$). Least squares means for copy number per microliter in serum QC-RT-PCR results for replicates 2, 3, and 4 at 10 d after infection were $10^{7.91 \pm 0.12}$ for the $FACS^{hi}$ line and $10^{8.27 \pm 0.12}$ for the $FACS^{lo}$ line ($P < 0.06$; Figure 2). Serum levels of virus at 10 d after infection were correlated with the FACS results at r = –0.32 ($P < 0.001$) for $TCID_{50}$/mL and r = –0.25 ($P < 0.003$) for QC-RT-PCR copies per microliter. Virus titers in serum and BAL had begun to fall by 21 d after infection, at which point some had dropped below detectable levels. However, the least squares means for $TCID_{50}$/mL of serum virus titer at 21 d after infection were $10^{1.54 \pm 0.28}$ for the $FACS^{hi}$ line and $10^{0.80 \pm 0.28}$ for the $FACS^{lo}$ line ($P < 0.08$). Least squares means for serum QC-RT-PCR results in copies per microliter for replicates 2, 3, and 4 at 21 d after infection were $10^{6.51 \pm 0.50}$ for the $FACS^{hi}$ line and $10^{5.64 \pm 0.48}$ for the $FACS^{lo}$ line; however, the difference in copy number was not significant (Figure 3). Neither the $TCID_{50}$ titer nor the QC-RT-PCR copy number in serum at 21 d after infection was significantly correlated with the in vitro FACS results.

DISCUSSION

Previous work in our laboratory using an in vitro FACS assay demonstrated significant differences in the susceptibility of MDM to PRRSV in culture between commercial pig lines (Vincent et al., 2005). The purpose of this investigation was to determine whether the differences demonstrated in vitro between the $FACS^{hi}$ and $FACS^{lo}$ lines were correlated with disease traits in vivo.

Significant differences between the lines were found for five variables related to disease severity during the acute period, including respiratory score sum, number of febrile days, virus levels in serum, and macroscopic and microscopic lung scores. Of these traits, the 10 d after infection respiratory score sum, number of febrile days, virus levels in serum, and macroscopic lung score were significantly, but negatively, correlated with the in vitro MDM-FACS assay. The FACS^{lo} line was consistently more susceptible to PRRSV in the early time points, although clinically the differences were small. Interestingly, at 21 d after infection, the relationship reversed, and the FACS^{lo} line had less viremia as measured by a tissue culture titration method and viral RNA copy number in the serum as quantified by QC-RT-PCR. It also seems that the differences in the line means were increasing at 21 d after infection. Because PRRSV has been well documented to establish chronic, long-term infections in a large percentage of pigs (Horter et al., 2002; Wills et al., 2003), the results at 21 d after infection may be indicative of a host genetic component in the length of viremia, although later time points would be required to support this. The existence of chronically infected animals in farms attempting to control or eradicate PRRSV is currently a critical issue in the swine industry. A significant difference between the two lines also was demonstrated for 21 d after infection for microscopic lung score; the FACS^{lo} line had a slightly greater interstitial pneumonia score. The increased pneumonia score at this latter time point may be attributed to the severity of the lesions during the acute stage of the disease, although the relationship between microscopic pneumonia score at 10 and 21 d after infection is unknown at this point.

Reports in the literature on differences in susceptibility to PRRSV in the host have been limited, although anecdotal reports from field observations suggest wide ranges in clinical disease within and between farms. An experimental challenge study using pigs from Duroc, Hampshire, and Meishan breeds demonstrated differences in PRRSV pathology among breeds, most notably that Hampshire pigs had significantly greater PRRSV-induced macroscopic lung lesions (Halbur et al., 1998). In another study, additive genetic variation in susceptibility to respiratory diseases was detected in pigs from Yorkshire, Duroc, Landrace, and Hampshire breeds; Yorkshire and Duroc breeds showed an increase in general resistance to clinical and subclinical respiratory disease (Henryon et al., 2001). However, the characterization of the respiratory disease and inciting pathogen were not individually evaluated. The limited reports in the literature on genetic susceptibility to PRRSV have investigated differences between purebred lines, and no evidence has been presented for differences in commercial crossbred lines used as breeding stock in a large number of swine operations around the world. Based on the in vitro and in vivo results described here, variability between commercial genetic lines in susceptibility to PRRSV seems to exist.

It has been shown that PRRSV has a restricted tropism for some populations of macrophages, and different stages of activation can influence their susceptibility to the virus (Duan et al., 1997). Because the macrophage is a potent effector cell of the immune response, it is not clear what proportion of the infected macrophages in vivo would support propagation of virus and/or stimulate an immune response through autocrine or paracrine activation. Studies investigating the pathways for PRRSV infection have shown that virus attachment occurred in nearly all alveolar macrophages cultured with virus within 60 min; however, viral replication only seemed to occur in 30% of the cells (Nauwynck et al., 1999). In addition, PRRSV has been shown to induce apoptosis primarily in uninfected bystander macrophages in vivo and in vitro (Sirinarumitr et al., 1998; Labarque et al., 2003), which may be a protective mechanism by the host to prevent subsequent infection of incoming monocytes by infected resident macrophages. Porcine reproductive and respiratory syndrome virus has been shown to use sialoadhesin present on the surface of macrophages for virus entry through clathrin-mediated endocytosis. However, the transfection of the sialoadhesin gene into a nonmacrophage cell type alone was insufficient to support uncoating and replication in the transfected cells (Vanderheijden et al., 2003), indicating additional steps in the infectious pathway. All of these factors suggest that host resistance to PRRSV is complex, and a more complete understanding of the molecular pathogenesis of PRRSV may reveal the biological basis for the relationship between the in vitro assay and the disease traits.

The in vitro assay was shown to be inversely correlated with respiratory score sum at 1 to 10 d after infection, number of febrile days from d 1 to 10 after infection, macroscopic lung lesion scores at d 10 after infection, and virus titer in serum at d 10 after infection. It should be noted that this relationship was investigated for one time point in vitro at 24 h after infection. This time point was chosen because of the limited number of MDM available when produced from peripheral monocytes and the PRRSV-induced apoptosis that occurs within 24 h after infection in culture (Sirinarumitr et al., 1998). This was balanced with the demonstration of peak infection levels of MDM by flow cytometry between 24 and 48 h after infection (Thacker et al., 1998). The difference in the susceptibility in the macrophages between the 2 lines at this early time point may indicate a difference in viral attachment, entry, or early stages of replication without time for many host response systems to be activated. The differences also may be related to differences in the activation and/or maturation of the MDM while in culture because it has been demonstrated that different activation and differentiation stages of macrophages affect their susceptibility to PRRSV (Duan et al., 1997). If this plays a role in the in vivo susceptibility of the host, a possible explanation for the switch in advantage in virus levels in the serum between the lines at the 10 and 21 d after infection

time points is that inherent differences in macrophages in their susceptibility to PRRSV infection and their ability to respond to activation may provide some benefit early on in infection. However, as more macrophages are recruited to the lung, the virus is able to persist in the newly recruited susceptible population of macrophages. Much work remains to provide direct evidence for this hypothesis.

The PRRSV-MDM FACS assay was not significantly correlated with susceptibility on an individual animal basis or even within an individual replicate, as was demonstrated by the variation in the assay between replicates (Vincent et al., 2005). This variation is most likely due to the inherent inconsistencies that are characteristic of biologic assays and the limited number of commercially available reagents necessary to characterize fully the phenotype of the porcine macrophages in culture. To remove assay-to-assay variation, it would be beneficial to incorporate an index macrophage cell line to control for changes in culture conditions and infectivity of the virus. To date, however, continuous macrophage lines have not been shown to support PRRSV infection (Weingartl et al., 2002), and the mechanism of infection for the permissive MARC-145 cells has been shown to be quite different than that of macrophages (Nauwynck et al., 1999). This suggests it also would not be a suitable index cell line for the PRRSV FACS assay. It also is important to note that each replicate had a different composite of sires to increase the representation of the genetic pool within the two lines. Although there was not a clear sire effect identified in the statistical analyses, this might have contributed to the variation noted among replicates in the magnitude of responses, both in vitro and in vivo.

Assay variation on an individual animal basis also is likely affected by the immune status of the pig and age of testing, in addition to the inherent variability in the assay itself. The health status of the animals used in this study changed over time between replicates, but no other alternative animals could be used because of the requirements of specific genetic lines and PRRSV-free status. Study animals from both lines tested positive for *Bordetella bronchiseptica*, *Pasteurella multocida*, *Mycoplasma hyorhinis*, and porcine respiratory coronavirus. Lesions indicative of *Mycoplasma hyopneumoniae* and cytomegalovirus also were present, but the organisms were not isolated. The presence of these organisms is unlikely to have interfered significantly with the health status and thereby the differences between the lines following PRRSV challenge because the 2 lines originated from the same source farm and were mixed as pen-mates in the same isolation room. The negative control animals in each of the replicates exhibited no respiratory disease caused by these other organisms. The health status of the challenge groups was relatively consistent, and the presence of other organisms may represent a PRRSV challenge more typical of field cases.

Identification of lines with decreased susceptibility to PRRSV-induced disease would be of great value to the swine industry because PRRSV has a major economic effect, and it has been difficult to manage through traditional methods that include vaccination and biosecurity measures. Highly virulent strains of PRRSV can cause extreme losses because of decreased reproductive performance in adult animals and impaired growth and increased mortality in growing animals. In addition, PRRSV has been demonstrated to have an immunomodulatory effect through decreased vaccine efficacy to *M. hyopneumoniae* (Thacker et al., 2000) and swine influenza virus (Thacker et al., 2003). Accordingly, low virulence strains also may cause economic losses because of impaired immune responses to concurrent or subsequent infections.

The results reported here and elsewhere suggest that selection of pigs that have increased resistance to this economically important disease should be considered. Genetic improvements in PRRSV resistance could be further advanced by identification of genes or genetic markers correlated with resistance. Monoclonal antibodies have been used to identify and characterize the porcine sialoadhesin molecule as a receptor for PRRSV entry into tissue macrophages (Vanderheijden et al., 2003). Although transfection of the sialoadhesin gene alone was not sufficient for viral replication, the characterization of this gene may lead to potential sources of genetic variation in susceptibility to PRRSV. Immune responses through regulation of cell surface molecules, including major histocompatibility complex and cytokine receptors, production of cytokines and chemokines, and production of a neutralizing antibody are other potential mechanisms in which genetic selection could be used to improve the health status of swine (Warner et al., 1987; Gogolin-Ewens et al., 1990; Edfors-Lilja et al., 1998). Alternatively, the existence of lines with large differences in susceptibility may allow for development of line crosses that would facilitate genomic scans to identify chromosomal regions associated with resistance to PRRSV. If chromosomal regions can be identified for PRRSV susceptibility, development of genetic markers to aid in the selection of animals with increased resistance may be feasible (Stear et al., 2001; van der Waaij et al., 2002).

Increasing our understanding of the pathogenesis of PRRSV and the molecules involved in regulating the specific immune responses taking place in the host will be beneficial in identifying the specific genes involved. Additionally, the identification of the host genes involved could potentially be used to develop genetic markers that improve health traits of pigs. Such an understanding is fundamental for improving preventative and therapeutic treatments against this important pig pathogen.

IMPLICATIONS

Porcine reproductive and respiratory syndrome is considered the most important disease affecting the

swine industry. Measures to control this pathogen have been largely unsuccessful, making selection of pigs with increased resistance highly attractive. This study evaluated two lines from a commercial breeding herd selected for study based on an in vitro screening assay. Differences were noted between the lines during the acute phase of the respiratory form of the disease, although the differences for many of the traits were small. At 21 d after infection, difference was observed in serum virus levels; however, the line with the advantage during the acute period had greater virus levels at the later time point, indicating that the interaction between host and virus is quite complex. Genetic variation in susceptibility to the virus seems to exist in crossbred commercial pig populations, and further study is warranted to investigate selection strategies to increase resistance to this important pathogen.

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