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# Differential immunity in pigs with high and low responses to porcine reproductive and respiratory syndrome virus infection<sup>1,2</sup>

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**ABSTRACT:** One hundred Hampshire × Duroc cross-bred pigs (HD) and 100 NE Index line (I) pigs were infected with porcine reproductive and respiratory syndrome (PRRS) virus and evaluated for resistance/susceptibility. Controls (100/line) were uninfected littermates to the infected pigs. Viremia, change in weight (WTA), and rectal temperature at 0, 4, 7, and 14 d postinfection were recorded. Lung, bronchial lymph node (BLN), and blood tissue were collected at necropsy (14 d postinfection). The first principal component from principal component analyses of all variables was used to rank the pigs for phenotypic response to PRRS virus. Low responders (low PRRS burden) had high WTA, low viremia, and few lung lesions; high responders (high PRRS burden) had low WTA, high viremia, and many lesions. The RNA was extracted from lung and BLN tissue of the 7 highest and 7 lowest responders per line

and from each of their littermates. Expression of 11 innate and T helper 1 immune markers was evaluated with cDNA in a 2 × 2 × 2 factorial design. Significant upregulation in lung, lymph, or both of infected pigs relative to controls occurred for all but one gene. Expression differences were greater in HD than I pigs. Significant downregulation for certain immune genes in low pigs, relative to littermate controls, was detected in lung and BLN, particularly in line I. Serum levels of the immune cytokines affirmed the gene expression differences. High preinfection serum levels of IL 8 were significantly associated with PRRS virus-resistant, low pigs. After infection, low expression of interferon gamma in cDNA and in serum was also correlated with PRRS virus resistance. Important genetic associations were revealed for fine mapping of candidate genes for PRRS virus resistance and determining the causative alleles.

**Key words:** gene expression, immune response, pig, porcine reproductive and respiratory syndrome virus resistance

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## INTRODUCTION

The porcine reproductive and respiratory syndrome virus (PRRSV) causes porcine reproductive and respiratory syndrome, an economically important disease of

pigs (Neumann et al., 2005) characterized by reproductive failure, interstitial pneumonia, and weak piglets. The virus targets alveolar macrophages (Murtaugh et al., 2002; Osorio, 2002) and induces apoptosis, resulting in ineffective elimination of the virus and persistence for several weeks (Labarque et al., 2003; Rowland et al., 2003; Chang et al., 2005). Clinical signs are more severe when coinfection with another pathogen occurs (Thacker et al., 2001; Thanawongnuwech et al., 2004).

Ample evidence for genetic variation in pigs in response to pathogens exists. Breed differences and additive genetic variation in incidences of atrophic rhinitis, respiratory diseases, and enteric diseases have been reported (Lundeheim, 1979, 1988; Van Diemen et al., 2002). Incidence of pigs in disease classifications and distributions of leukocytes in serum of pigs are low to moderately heritable (Henryon et al., 2001, 2002). Genetic variation in response to modulation of the immune system also has been demonstrated (Edfors-Lilja

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**Table 1.** Innate and acquired immunity genes evaluated

Gene symbol	Molecule name	Immune role <sup>1</sup>	Cytogenic/linkage map location
IFNA	Interferon – alpha	Innate, antiviral response	1q25
IL1B	Interleukin 1 beta	Innate; inflammatory	3q11-q14
IL6	Interleukin 6	Innate; proinflammatory	9q14-q15
IL8	Interleukin 8 (CXCL8)	Innate; activation	8:61.7 cM
CSF2	Colony stimulating factor 2	Innate; induction	None
IFNG	Interferon gamma	Th1; antiviral activity	5p12-q11
IL12B	Interleukin 12 beta	Th1; development	None
IL15	Interleukin 15	Th1; activation, proliferation	None
STAT1	Signal transducer and activator of transcription1	Th1; immunoregulation	15q23-q26
TNF- $\alpha$	Tumor necrosis factor	Innate/Th1; cell death, proinflammatory	7p11
IL10	Interleukin 10	T regulator; immunoregulation	9:80.3 cM
RPL32	Ribosomal protein L32	none; control	

<sup>1</sup>The cytogenetic map for the pig, based on data from 2002, can be accessed at <http://www.toulouse.inra.fr/lgc/pig/cyto.htm> (last accessed Apr. 2007).

et al., 1995; Mallard et al., 1998; Wilkie and Mallard, 1999).

Breeds and lines of pigs responded differently to infection with PRRSV (Halbur et al., 1998; Lowe et al., 2005; Petry et al., 2005; Vincent et al., 2006), indicating genetic variation exists. However, because most nucleus swine breeding populations are maintained with very high health status, selection for resistance to many pathogens that seriously affect commercial herds using quantitative methods may not be practical. Selection using genetic markers or traits that can be measured in uninfected pigs will likely be more practical.

The purpose of this experiment was to characterize genetic differences in the pigs used in the PRRSV infection experiment reported by Petry et al. (2005). Lung and bronchial lymph tissue were used to determine differences in expression of specific immune function genes and levels of cytokines between pigs classified as more resistant or more susceptible to PRRSV infection.

## MATERIALS AND METHODS

This research was approved by the University of Nebraska Animal Care and Use Committee.

### *PRRSV Infection Experiment*

A gene expression study was conducted with 56 pigs from a PRRSV-infection experiment involving a total of 400 pigs. Design and details of the experiment are given in Petry et al. (2005). Only an overview is presented here.

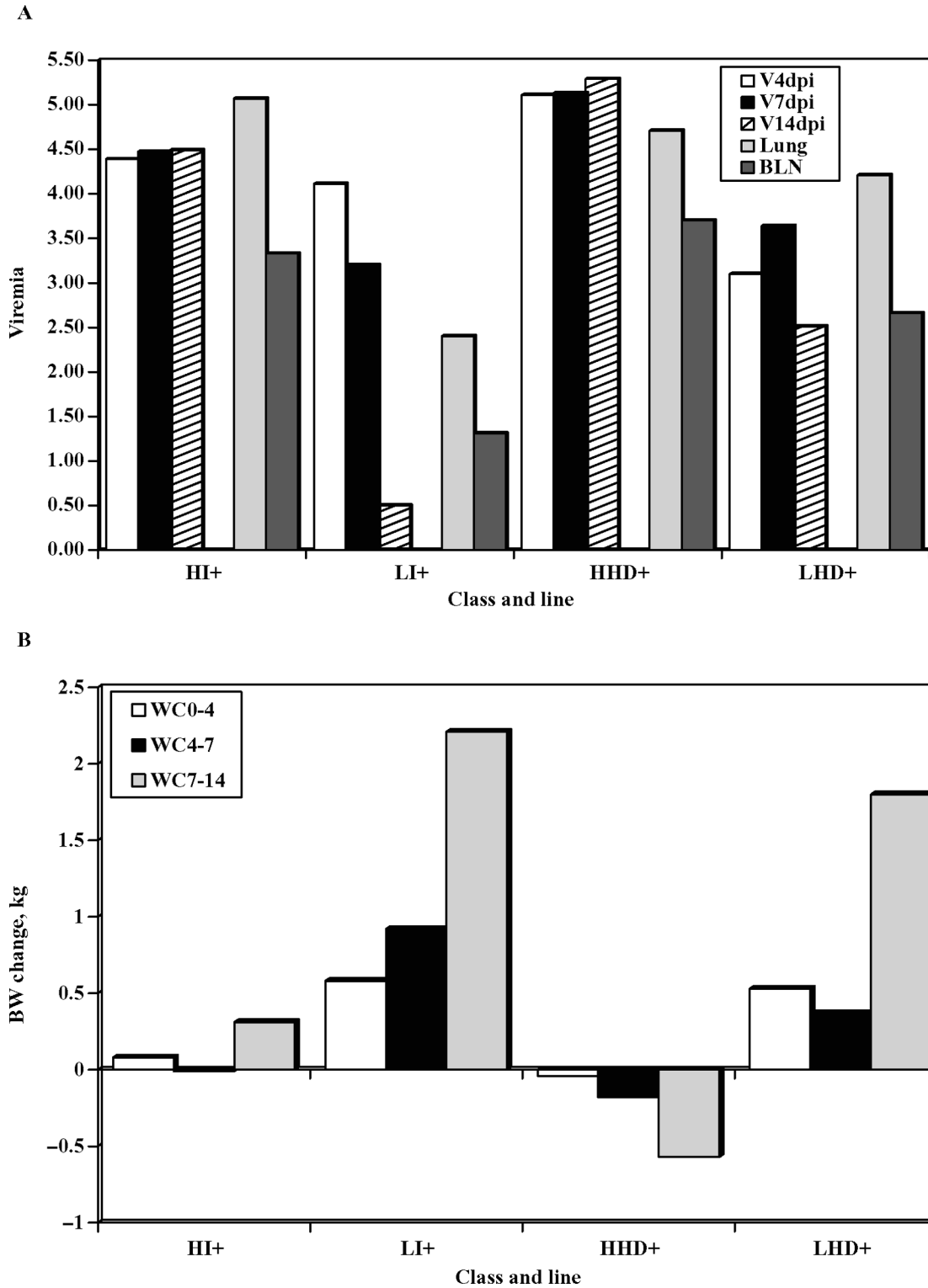
Two hundred pigs from the NE Index line (**I**), selected for 20 generations for increased litter size, and 200 pigs from a commercial Hampshire by Duroc (**HD**) cross, selected for lean growth were used in the PRRSV infection experiment. Johnson et al. (1999) described the selection history of line I. Responses in reproductive traits through generation 19 are given in Petry and Johnson (2004). A total of 200 pigs were infected with PRRSV and 200 uninfected littermates served as con-

trols. Pigs for the experiment were selected at random from the available litters, with 2 pigs of the same sex from as many litters and families as possible, representing a total of 83 sires and 163 dams. The experiment was conducted in 2 replicates within each of 2 seasons, with 50 pigs per breed in each year  $\times$  season  $\times$  replicate.

Pigs were housed in 2 isolation rooms of the University of Nebraska Animal Research Facility of the Veterinary and Biomedical Sciences Department, which are environmentally controlled facilities designed for disease research. Each room contained 2 pens, with line I pigs in 1 pen and line HD pigs in the other. There were 12 to 13 pigs per pen. Within each replication, 1 room was randomly assigned for treatment and the pigs in it were inoculated intranasally with 105 cell culture infectious dose 50% (**CCID**<sub>50</sub>) of PRRSV strain 97-7985 (Osorio et al., 2002). The application rate was 1 cc per nostril. Pigs in the other isolated room, which were littermates to those in the infected room, served as controls.

Phenotypic data included viremia from serum samples collected at 4, 7, and 14 d postinfection (**dpi**), changes in weight and rectal temperature from 0 to 4, 4 to 7, and 7 to 14 dpi, lung and bronchial lymph node viremia from tissue collected at necropsy at d 14, and severity of lung lesions. Blood serum at d 0 before infection was collected and stored. Shin and Molitor (2002) reported that more than 80% of infected pigs showed the peak level of viral RNA concentration in serum at 5 dpi and began to clear the virus from the systemic circulation thereafter. Work by Osorio et al. (2002) also indicated that early clinical signs of PRRSV are evident in the early postinoculation period and that viremic titers are at maximum by d 14. Therefore, samples were collected at 4, 7, and 14 dpi to monitor changes during and shortly after the acute phase of viral infection.

The I and HD pigs responded differently to infection with PRRSV (Petry et al., 2005), indicating genetic variation in the response to infection. Uninfected HD pigs gained more and had greater rectal temperature from



**Figure 1.** Mean viremia (panel A) in serum at 4, 7, and 14 d postinfection (V4 dpi, V7 dpi, and V14 dpi, respectively) and in lung and bronchial lymph node (BLN) and BW change (WC, kg; panel B) from d 0 to 4, d 4 to 7, or d 7 to 14 postinfection (dpi = day postinfection) for the 7 pigs of each line (I = Index, HD = Hampshire-Duroc) that were infected with PRRSV (+) and were classified as having high (H) or low (L) disease burden by the principal component analysis.

0 to 14 dpi than uninfected I pigs, whereas infected I pigs gained more and had lower rectal temperature than infected HD pigs. Viremia ( $CCID_{50}/mL$ ) was also greater in HD than I pigs at 4, 7, and 14 dpi. Viremia

differences in lung and bronchial lymph nodes were not significant but tended to be greater in HD than I pigs.

Based on these results, the current experiment was designed to determine whether expression of certain

**Table 2.** Overall line means and SD, and means for the 7 high and low responders for NE Index line (I) and Hampshire-Duroc (HD) crossbred pigs infected with PRRSV (+)

Trait <sup>1</sup>	Line I				Line HD			
	$\mu^2$	$\sigma^3$	HI+ <sup>4</sup>	LI+ <sup>5</sup>	$\mu^2$	$\sigma^3$	HHD+ <sup>6</sup>	LHD+ <sup>7</sup>
V4	4.17	0.60	4.39	4.11	4.54	0.82	5.11	3.10
V7	3.91	0.76	4.47	3.20	4.40	0.70	5.13	3.64
V14	3.00	1.21	4.49	0.50	3.59	0.99	5.29	2.51
WC <sub>0-4</sub>	0.32	0.39	0.08	0.58	0.29	0.34	-0.04	0.53
WC <sub>4-7</sub>	0.33	0.41	-0.01	0.92	0.06	0.33	-0.18	0.37
WC <sub>7-14</sub>	1.35	0.79	0.31	2.21	0.71	0.78	-0.57	1.80
TC <sub>0-4</sub>	0.92	1.56	1.09	0.33	1.76	1.46	0.79	1.94
TC <sub>4-7</sub>	0.58	1.25	1.00	0.66	0.82	1.70	1.36	-0.06
TC <sub>7-14</sub>	-0.16	1.17	0.36	-1.01	-0.36	1.63	-2.84	-0.26
LV	3.96	1.28	5.07	2.40	4.45	0.78	4.71	4.21
LNV	2.55	1.30	3.33	1.31	3.12	0.99	3.70	2.66
L	1.26	0.79	1.57	1.00	1.96	0.57	1.57	2.00

<sup>1</sup>V4, V7, and V14 = viremia titer, expressed as  $\log_{10}$  of CCID<sub>50</sub>/mL, (CCID<sub>50</sub> = cell culture infectious dose 50%) in serum collected at 4, 7, and 14 d, respectively (SEM = 0.07); WC<sub>0-4</sub>, WC<sub>4-7</sub>, and WC<sub>7-14</sub> = weight change, in kg, from d 0 to 4, d 4 to 7, and d 7 to 14, respectively (SEM = 0.05); TC<sub>0-4</sub>, TC<sub>4-7</sub>, and TC<sub>7-14</sub> = temperature change, in °C, from d 0 to 4, d 4 to 7, and d 7 to 14, respectively (SEM = 0.10); LV and LNV = viral titer,  $\log_{10}$  CCID<sub>50</sub>/mL, in lung (SEM = 0.11) and bronchial lymph (SEM = 0.07), respectively, collected at necropsy on d 14; L = severity score of lung lesions (SEM = 0.12).

<sup>2</sup> $\mu$  = line mean of pigs infected with PRRSV.

<sup>3</sup> $\sigma$  = line SD of pigs infected with PRRSV.

<sup>4</sup>HI+ = mean of 7 I pigs with high response to PRRSV infection.

<sup>5</sup>LI+ = mean of 7 I pigs with low response to PRRSV infection.

<sup>6</sup>HHD+ = mean of 7 HD pigs with high response to PRRSV infection.

<sup>7</sup>LHD+ = mean of 7 HD pigs with low response to PRRSV infection.

immune function genes differed between pigs in the tails of the response distribution. Phenotypic data for infected pigs were subjected to clustering and principal component (PC) procedures to identify 28 pigs, 7 pigs within each line in the outermost tails (high and low) of the distributions of the viral response variables. The 28 control littermates to each of these pigs also were selected, resulting in 56 pigs used in the current gene expression experiment.

With the exception of 2 pigs, PC and clustering identified the same pigs in the upper and lower tails of the distribution within each population. Therefore, only results of the PC analysis are presented. The first PC eigenvector, which accounted for 27% of the variation, was used to rank the pigs. Pigs with the greatest PC values were classified as having high viremia and high symptoms of PRRS (high); pigs with low PC values were classified as having low viremia and low symptoms (low).

A  $2 \times 2 \times 2$  factorial treatment design was utilized for the gene expression experiment. The design effects included class (high or low based on the PC analysis), line (I or HD), and treatment (infected or uninfected), with 7 pigs in each of the 8 treatment groups. After the 56 pigs were identified, the data reported by Petry et al. (2005) were used to confirm that characterization into high and low classes was not confounded with other types of infection.

### Tissue Storage and RNA Preparation

Lung and bronchial lymph node (BLN) tissue were collected at necropsy, placed in Optimal Cutting Tem-

perature Compound (Sakura Finetec US Inc., Torrance, CA), snap frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$ . The RNA from the tissues of the 56 pigs identified by PC analysis was extracted with Trizol, as previously described (Royae et al., 2004; Dawson et al., 2005). Integrity, quantity, and quality of RNA were assessed using the Agilent Bioanalyzer 2100 and RNA 6000 Labchip Kit (Agilent Technologies, Palo Alto, CA). The RNA was treated with DNase before cDNA synthesis using the Superscript reverse transcription (Invitrogen, Carlsbad, CA) and oligo-dT. The reverse transcription-PCR primers and TaqMan probes were designed with Primer Express (Applied Biosystems, Foster City, CA) and manufactured by BioSource (Dawson et al., 2005). More details on the assay design are given at [www.ars.usda.gov/Services/docs.htm?docid=6065](http://www.ars.usda.gov/Services/docs.htm?docid=6065) (last accessed 8 May 2007). Normalization of the samples was performed by using a standard amount of RNA (10  $\mu\text{g}$ ) for every sample for the cDNA production and confirmed by the cycle threshold (Ct) value for the housekeeping gene RPL32. The reverse transcription-PCR analyses were conducted in duplicate on 100 ng of cDNA/25  $\mu\text{L}$  reaction for each well utilizing the Brilliant Kit (Stratagene, La Jolla, CA) and an Applied Biosystems PRISM 7700 Sequence Detector System. The conditions used for amplification were  $50^{\circ}\text{C}$  for 2 min;  $95^{\circ}\text{C}$  for 10 min; 40 cycles of  $95^{\circ}\text{C}$  for 15 s,  $60^{\circ}\text{C}$  for 1 min; and then remaining at  $4^{\circ}\text{C}$ .

### Gene Expression Analyses

Eleven immune genes involved in innate and acquired immunity and 1 housekeeping gene were evalu-

**Table 3.** Significance levels for fixed effects on expression of genes (cycle threshold value) in lung and bronchial lymph node tissue at 14 d postinfection

Trait <sup>1</sup>	Class <sup>2</sup>	Line <sup>3</sup>	Class × line	Treatment	Treatment × class	Line × treatment	Line × treatment × class
<b>Lung</b>							
IFNA							
IFNG	***			***		***	
IL15							
RPL32							
STAT1	**			***		*	
IL1B				***	**		*
IL12B			***	***	***	***	
CSF2				***	**		
IL8				***	**	**	
IL10	***						
IL6							
TNF- $\alpha$				**		**	
<b>Bronchial lymph node tissue</b>							
IFNA		*		***		***	
IFNG			**	***	**	*	
IL15							
RPL32					*		
STAT1				***			
IL1B				**	**		
IL12B				**			
CSF2		***		***			
IL8				***	***		
IL10			*	**	**		
IL6			**	**			
TNF- $\alpha$		*		***	*		

<sup>1</sup>IFNA = interferon alpha; IFNG = interferon gamma; IL15 = IL 15; RPL32 = ribosomal protein L32; STAT1 = signal transducer and activator of transcription 1-alpha; IL1B = IL 1 beta; IL12B = IL 12 beta; CSF2 = colony stimulating factor 2; and TNF- $\alpha$  = tumor necrosis factor alpha.

<sup>2</sup>Class = high or low responders based on the principal component analysis.

<sup>3</sup>Line = NE Index line or Hampshire-Duroc crossbred pigs.

\* $P < 0.10$ ; \*\* $P < 0.05$ ; \*\*\* $P < 0.01$ .

ated (Table 1). Gene expression Ct values were recorded with the Applied Biosystems PRISM 7700 Sequence Detector System. The Ct value is the intersection between the gene amplification plot and the threshold, defined as 10 times the SD of the background fluorescence intensity measured between cycles 3 and 15. The Ct value is directly related to the amount of PCR product and therefore to the initial amount of target cDNA present in the PCR reaction. Samples producing high Ct values had less cDNA than samples producing low values because more PCR cycles were needed to reach the threshold.

### Serum Cytokine Protein Analyses

Cytokine protein levels in serum were measured using commercial ELISA assays (Royae et al., 2004). The BioSource Immunoassay kit (Camarillo, CA) was used for porcine interferon gamma (IFNG) and IL 10 (IL10). The R&D Systems DuoSet ELISA Development kit (Minneapolis, MN) was used to measure pig IL 1 beta (IL1B), IL6, IL8, IL12/IL23p40, and tumor necrosis factor alpha (TNF- $\alpha$ ). The d 14 sera were tested 3 months before testing of sera from the other time points and with different batches of reagents. Aliquots of sera

were tested at several dilutions to obtain accurate cytokine protein levels.

### Statistical Analysis

The SAS software (SAS Inst., Inc., Cary, NC) was used for all analyses. The data from duplicate Ct values were run through the JMP 5.0.1 Student's *t*-test. The difference between means (sample vs. control) was then used to determine fold-values. The Ct values were analyzed with a mixed model, with class, line, treatment, and all possible interactions as fixed effects. Litter within class  $\times$  line was treated as a random effect. Age was fitted as a covariate for the gene expression values to adjust the records to the same age. Cytokine protein levels were analyzed with a similar model, except that day and all its interactions were added to the fixed effects.

Correlations among expression patterns within tissue and across tissues were calculated with the MANOVA option of SAS, including line, class, treatment, and interactions in the model. Correlations among cytokine protein levels were calculated with the same procedure.

**Table 4.** Least squares (cycle threshold value) means for expression of genes in lung and bronchial lymph node (BLN) tissue

Item <sup>2</sup>	Group <sup>1</sup>							
	HHI-	LLI-	HHI+	LLI+	HHHD-	LLHD-	HHHD+	LLHD+
IFNA lung	29.25	28.66	29.73	29.18	28.59	30.59	27.87	30.18
IFNA BLN	30.14	30.56	30.03	30.51	29.07	29.60	30.12	30.84
IFNG lung	26.42	28.09	24.69	27.62	27.58	28.62	23.97	25.84
IFNG BLN	24.84	25.58	22.39	24.75	26.49	25.26	22.90	23.40
IL15 lung	30.97	31.68	30.77	32.11	30.68	31.49	29.89	31.56
IL15 BLN	29.07	29.99	28.24	30.94	29.41	30.13	29.04	29.80
RPL32 lung	17.53	17.42	17.17	17.48	17.29	17.54	16.83	17.60
RPL32 BLN	16.48	16.11	16.12	16.11	16.64	16.00	16.20	16.13
STAT1 lung	20.63	21.45	19.80	21.75	20.88	21.81	19.36	20.58
STAT1 BLN	18.61	18.79	17.37	18.50	18.71	18.54	17.75	17.74
IL1B lung	27.64	26.34	24.80	26.34	27.47	28.36	24.67	25.89
IL1B BLN	27.27	26.64	25.25	27.27	26.94	25.79	25.65	26.95
IL12B lung	32.32	30.21	30.99	30.77	32.09	32.17	29.66	30.49
IL12B BLN	26.75	26.76	26.06	26.30	28.06	26.73	26.55	26.63
CSF2 lung	28.38	27.26	26.89	27.37	27.68	27.88	25.80	26.74
CSF2 BLN	30.26	29.57	30.72	30.75	30.81	30.55	31.56	31.08
IL8 lung	25.03	24.73	22.48	23.99	25.04	25.81	21.51	23.59
IL8 BLN	26.33	26.04	22.33	25.72	26.64	24.97	23.45	24.39
IL10 lung	25.40	27.47	24.49	27.84	25.50	26.93	24.77	26.48
IL10 BLN	23.51	24.59	22.27	24.86	24.38	24.13	22.93	23.85
IL6 lung	26.39	27.01	26.05	27.04	26.18	26.56	26.10	26.72
IL6 BLN	24.41	25.16	23.47	24.81	25.13	24.10	24.22	24.08
TNF- $\alpha$ lung	27.85	28.00	27.45	28.63	28.41	29.14	26.54	28.07
TNF- $\alpha$ BLN	26.06	25.86	25.08	25.48	26.95	25.87	25.88	25.63

<sup>1</sup>HHI- = High viremia, high symptoms uninfected NE Index line (I) pig; LLI- = Low viremia, low symptoms uninfected NE Index line (I) pig; HHI+ = High viremia, high symptoms infected NE Index line (I) pig; LLI+ = Low viremia, low symptoms infected NE Index line (I) pig; HHHD- = High viremia, high symptoms uninfected Hampshire-Duroc (HD) crossbred pig; LLHD- = Low viremia, low symptoms uninfected Hampshire-Duroc (HD) crossbred pig; HHHD+ = High viremia, high symptoms infected Hampshire-Duroc (HD) crossbred pig; and LLHD+ = Low viremia, low symptoms infected Hampshire-Duroc (HD) crossbred pig.

<sup>2</sup>IFNA = interferon alpha; IFNG = interferon gamma; IL15 = IL 15; RPL32 = ribosomal protein L32; STAT1 = signal transducer and activator of transcription 1-alpha; IL1B = IL 1 beta; IL12B = IL 12 beta; CSF2 = colony stimulating factor 2; and TNF- $\alpha$  = tumor necrosis factor alpha.

## RESULTS

In this experiment we investigated whether expression of certain immune function genes differed between pigs in the tails of the response distribution to infection with PRRSV. Pigs that had been infected with PRRSV were ranked on the first principal component of all variables recorded and the 7 with the greatest or lowest disease burden within each genetic line, were selected. Their littermates were included to determine whether expression differences were a response to virus or were innate differences between classes.

Viremia and weight changes for I and HD pigs that were infected with virus and then classified as having high and low disease burden are shown in Figure 1. Responses are similar, but more dramatic, than average responses for all pigs as reported by Petry et al. (2005). Pigs in the high-class of both lines had high serum viremia levels at each day (Figure 1, panel A), indicating high disease burden to the end of the evaluation period. Line I pigs in the low class, however, began clearing the virus by 7 dpi and had relatively low levels 14 dpi. The HD, high-class pigs responded somewhat

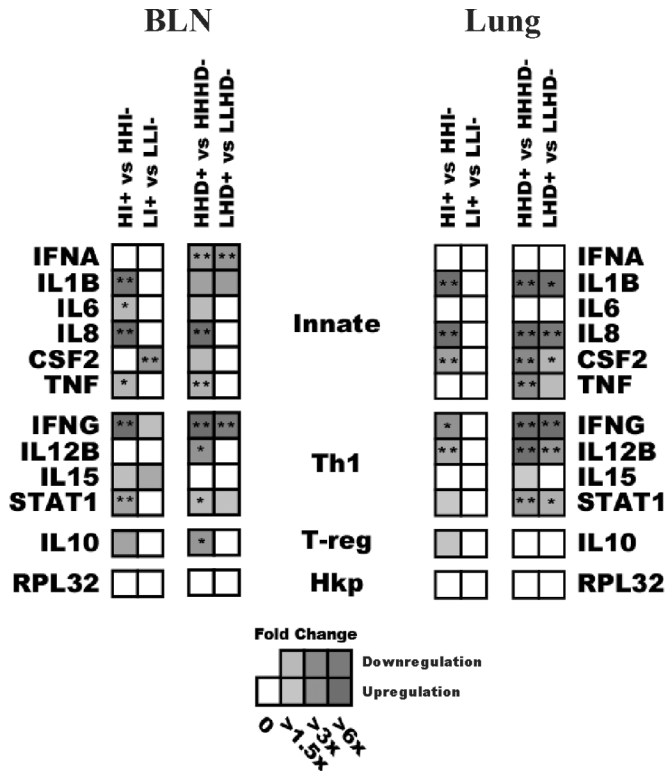
differently, having greatest levels of serum viremia 7 dpi, declining at 14 dpi, but not reaching the same low values of I, L-CLASS pigs.

Responses in weight gain (Figure 1, panel B) were consistent with responses in viremia. The high-class pigs either lost weight or gained very little weight whereas L-class pigs of both genetic lines gained weight during each period.

Table 2 contains line means and SD for all pigs that were infected with PRRSV and for the 7 infected high and low pigs of each line. All pigs challenged with virus became infected as indicated by the 4 dpi viremia values, but low-class pigs of both lines began to clear the virus more quickly and thus had less viremia at 7 and 14 dpi, whereas pigs in the high class had a continual increase in viremia from 4 to 14 dpi. Pigs in the low class also had increasing weight change after infection with PRRSV whereas pigs in the high class had minimal or even negative weight change.

**Gene Expression.** Significance values for effects in the models for gene expression Ct values are in Table 3. Least squares means are in Table 4. Figure 2 is an overview illustrating comparative expression values for





**Figure 2.** Comparative changes in immune gene expression 14 d after PRRSV infection, presented as the ratio of the Ct values of infected pigs to those of their uninfected littermates. Squares are color-coded (or shaded) to denote up- or downregulation of gene expression ( $*P \leq 0.05$ ,  $**P \leq 0.01$ ) between infected and uninfected controls.

control vs. PRRSV infected tissues. Overall, at 14 dpi, when the lung and BLN tissues were collected, the low pigs had completed their immune response and had low or no changes in immune gene expression as compared with the uninfected controls; high pigs however were still trying to control the infection and still exhibited changes in immune marker expression. Only in the lung were the low responders (LHD) pigs still expressing increased levels of immune genes; these responses were consistent with their high level of viremia in that tissue (Table 2). As expected, no differences in expression of RPL32, the housekeeping gene, between any fixed effects were detected in lung or BLN. Mean expression values of RPL32, 17.36 Ct in lung and 16.22 Ct in BLN, are baseline expression values.

### Innate Genes

**IFNA.** Expression of IFNA in lung did not differ between infected and uninfected pigs; however, a line  $\times$  treatment interaction ( $P < 0.01$ ) in expression of IFNA in BLN occurred (Table 3). The I pigs infected with PRRSV and their uninfected littermates had similar expression levels (Table 4), but uninfected HD pigs had

greater expression of IFNA than infected HD pigs ( $-1.15 \pm 0.28$  Ct).

**IL1B.** Interaction of class  $\times$  treatment in expression of IL1B in both lung ( $P < 0.05$ ) and BLN ( $P < 0.01$ ) existed (Table 3). Means are in Table 4 and are illustrated in Figure 3. High and low responders to PRRSV had greater expression levels of IL1B in lung than their uninfected littermates. The difference between high pigs and their uninfected littermates was  $-2.82 \pm 0.51$  Ct, and the difference between infected low pigs and their uninfected littermates was  $-1.23 \pm 0.51$  Ct. A different interaction occurred in BLN. Expression was greater in infected high pigs than in uninfected littermates ( $-1.65 \pm 0.49$  Ct), and lower in infected low pigs than in their uninfected littermates ( $0.90 \pm 0.49$  Ct).

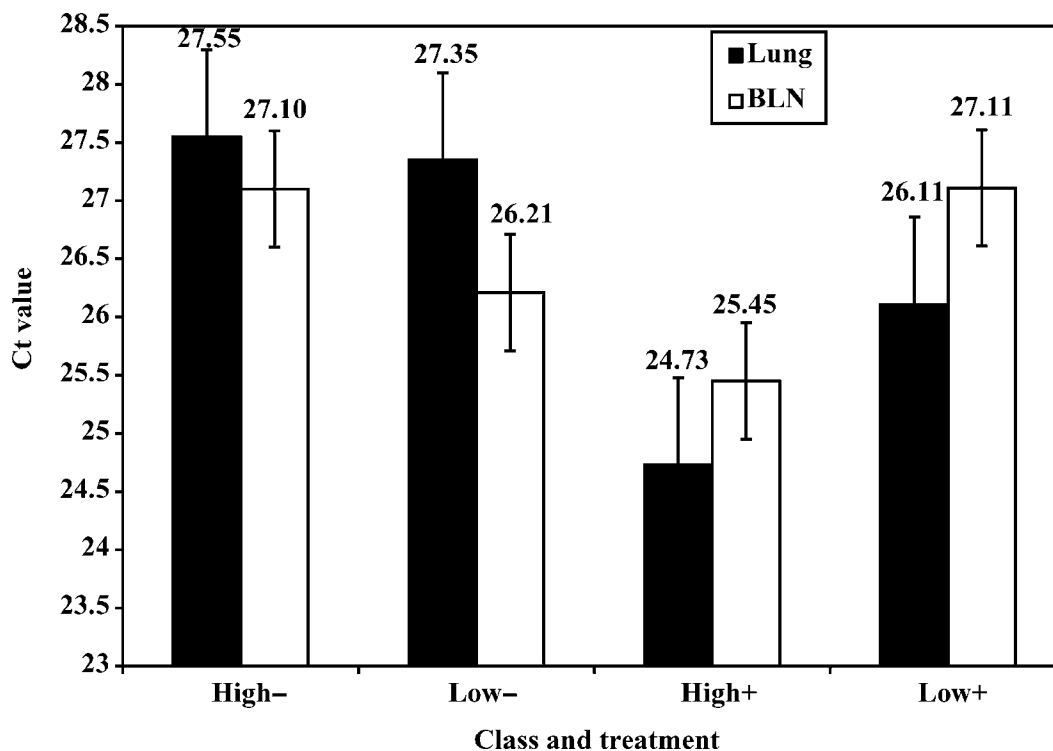
**IL6.** No significant differences among main effects in expression of IL6 in lung existed (Table 3); however, high pigs tended to have greater expression ( $-0.65 \pm 0.54$  Ct) than low pigs (Table 4). Both treatment and interaction of class with treatment affected ( $P < 0.05$ ) expression of IL6 in the BLN. Overall, infected pigs had greater expression than uninfected pigs ( $0.56 \pm 0.21$  Ct). High-class I pigs had greater expression than low pigs ( $-1.04 \pm 0.70$  Ct), however L-class HD pigs had greater expression than high-class pigs ( $-0.58 \pm 0.46$  Ct).

**IL8.** Pigs uninfected with PRRSV had less expression ( $P < 0.05$ ) of IL8 in lung than infected pigs (Table 3). Means are in Table 4 and are illustrated in Figure 4. Uninfected pigs in both classes had similar patterns of expression, but infected high pigs had greater expression than infected low pigs ( $-1.56 \pm 0.61$  Ct). Also, infected HD pigs at 14 dpi had greater expression of IL8 than infected I pigs ( $-1.25 \pm 0.61$  Ct), even though uninfected I pigs had greater expression than uninfected HD pigs. However, in the BLN, infected low pigs and their uninfected littermates had similar patterns of expression (low+ = 25.05 Ct vs. low- = 25.51 Ct;  $P = 0.50$ ), but infected high pigs had greater expression of IL8 than uninfected littermates ( $-3.59 \pm 0.66$  Ct).

**CSF2.** An interaction of class and treatment existed for expression of CSF2 in the lung (Tables 3 and 4). Infected and uninfected pigs in the low-class had similar expression values (27.06 Ct vs. 27.57 Ct) whereas infected high pigs had greater expression than uninfected littermates ( $-1.69 \pm 0.35$  Ct). No interactions in expression of CSF2 in the BLN were detected, however I pigs had greater expression than HD pigs ( $-0.67 \pm 0.25$  Ct) and infected pigs had greater expression than uninfected pigs ( $-0.73 \pm 0.20$  Ct).

### T Helper 1 Associated Genes

**IFNG.** A line  $\times$  treatment interaction in expression of IFNG in the lung existed (Table 3). The interaction is illustrated in Figure 5. Infected pigs of both lines had greater expression than uninfected littermates (I =  $-1.1$  Ct and HD =  $-3.2$  Ct), however infected HD pigs



**Figure 3.** Interleukin 1 beta (IL1B) expression level (cycle threshold value; Ct) in lung (SEM = 0.75) and bronchial lymph node (BLN, SEM = 0.50) tissue for pigs classified as having high or low disease burden by the principal component analysis and that were uninfected (-) or infected (+) with PRRSV (class  $\times$  treatment,  $P < 0.05$  and  $P < 0.01$  for lung and BLN, respectively).

had greater expression than infected I pigs ( $-2.10 \pm 0.67$  Ct). A class  $\times$  treatment and class  $\times$  line interaction occurred for expression of IFNG in the BLN (Figure 6). Uninfected pigs of high and low classes had similar expression levels (I = 25.42 Ct and HD = 25.67 Ct), however expression in infected high pigs at 14 dpi was greater than in infected low pigs ( $-1.68 \pm 0.62$  Ct). Both high- and low-class HD pigs had similar expression patterns in BLN (high responding HD pigs = 24.70 Ct vs. LHD = 24.33 Ct), whereas greater expression ( $1.55 \pm 0.85$  Ct) occurred in high-class I pigs than low-class I pigs.

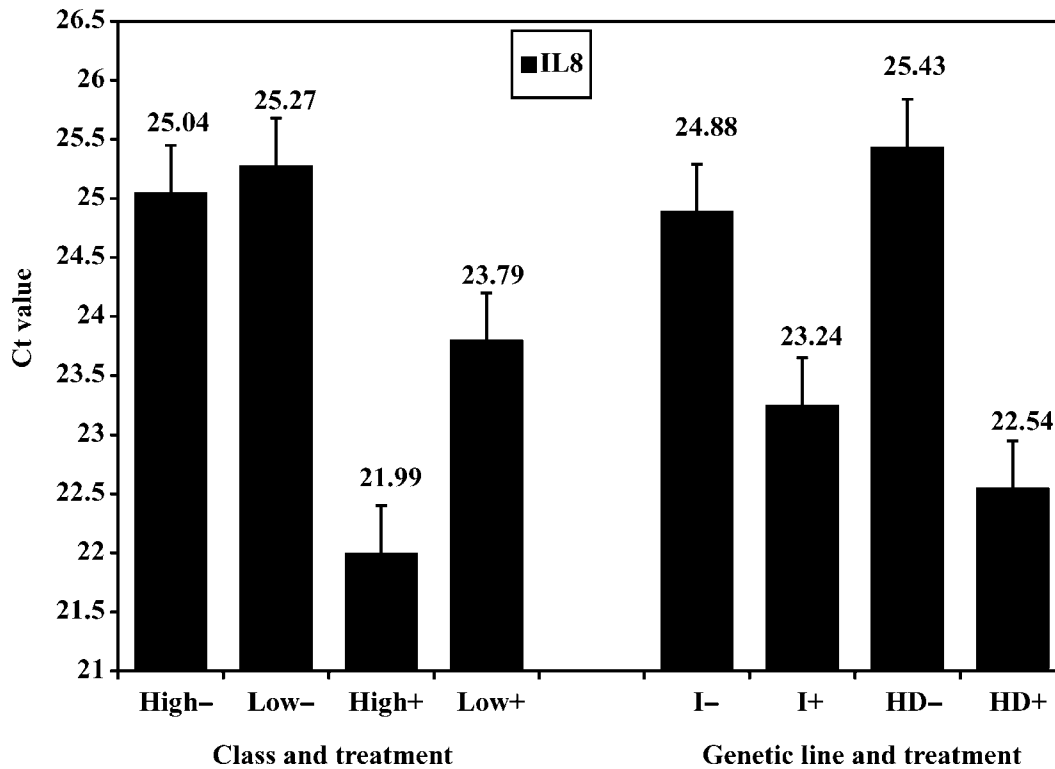
**IL12B.** Interactions in expression of IL12B in the lung included class  $\times$  line, class  $\times$  treatment, and line  $\times$  treatment ( $P < 0.01$ ; Table 3). Interactions are illustrated in Figure 7. High responding I pigs had less IL12B expression ( $1.17 \pm 0.68$  Ct) than low responding I pigs, whereas high responding HD pigs tended to have greater ( $-0.46 \pm 0.45$  Ct) expression than LHD. A similar class  $\times$  treatment response occurred in that uninfected high-class pigs had less expression than uninfected low-class pigs ( $1.01 \pm 0.55$  Ct), whereas infected high-class pigs had slightly greater lung IL12B expression than infected low-class pigs ( $-0.30 \pm 0.55$  Ct). The line  $\times$  treatment interaction existed because infected pigs had greater expression than uninfected pigs in both lines; however, the response in HD pigs was

greater than in I pigs ( $-1.68 \pm 0.43$  Ct). No interactions in expression of IL12B in BLN were detected, but infected pigs had greater expression than uninfected pigs ( $-0.69 \pm 0.29$  Ct).

**IL15.** No significant effects existed for expression of IL15 in the lung or BLN (Table 3). The high-class pigs tended to have greater expression than low-class pigs,  $-1.13 \pm 0.88$  Ct in lung and  $-1.27 \pm 0.75$  Ct in BLN, and HD pigs tended to have greater expression in lung than I pigs ( $-0.48 \pm 0.52$  Ct).

**STAT1.** Interactions in expression patterns of STAT1 in lung and BLN were not significant; however, a treatment effect occurred in both tissues, and class differences were significant in lung and BLN (Table 3). Expression of STAT1 in lung and BLN were similar. Infected pigs had greater expression in lung ( $-0.82 \pm 0.28$  Ct) and BLN ( $-0.82 \pm 0.21$  Ct) than uninfected pigs. Also, high-class pigs had greater expression in lung than low-class pigs ( $-1.23 \pm 0.59$  Ct).

**TNF- $\alpha$ .** A line  $\times$  treatment interaction ( $P < 0.01$ ) in expression of TNF- $\alpha$  in the lung existed (Table 3). Expression in infected and uninfected I pigs was similar; however, infected HD pigs had  $-1.59 \pm 0.57$  Ct greater expression than uninfected HD pigs. No interactions in BLN were detected. A treatment difference in expression of TNF- $\alpha$  in the BLN occurred (Table 3). Expression was greater in infected pigs than in unin-



**Figure 4.** Interleukin 8 (IL8) expression level (cycle threshold value; Ct) in lung (SEM 0.41) tissue for pigs classified as having high or low disease burden by the principal component analysis of the NE Index line (I) or Hampshire-Duroc (HD) crossbreds that were uninfected (-) or infected (+) with PRRSV (class  $\times$  treatment and line  $\times$  treatment,  $P < 0.05$ ).

infected littermates ( $-0.67 \pm 0.19$  Ct), which is consistent with the expression pattern in the lung.

### T Regulatory Gene

**IL10.** No interactions in expression of IL10 in the lung were detected (Table 3). The high pigs had greater expression ( $-2.14 \pm 0.60$  Ct) than low pigs. Line  $\times$  treatment interaction existed ( $P < 0.05$ ) for expression in the BLN (Figure 8). The expression pattern in the BLN was similar to the pattern in the lung in that high-class pigs had greater expression than low-class pigs; however, infected pigs had greater expression than uninfected pigs ( $-1.34 \pm 0.59$  Ct).

### Cytokine Protein Levels

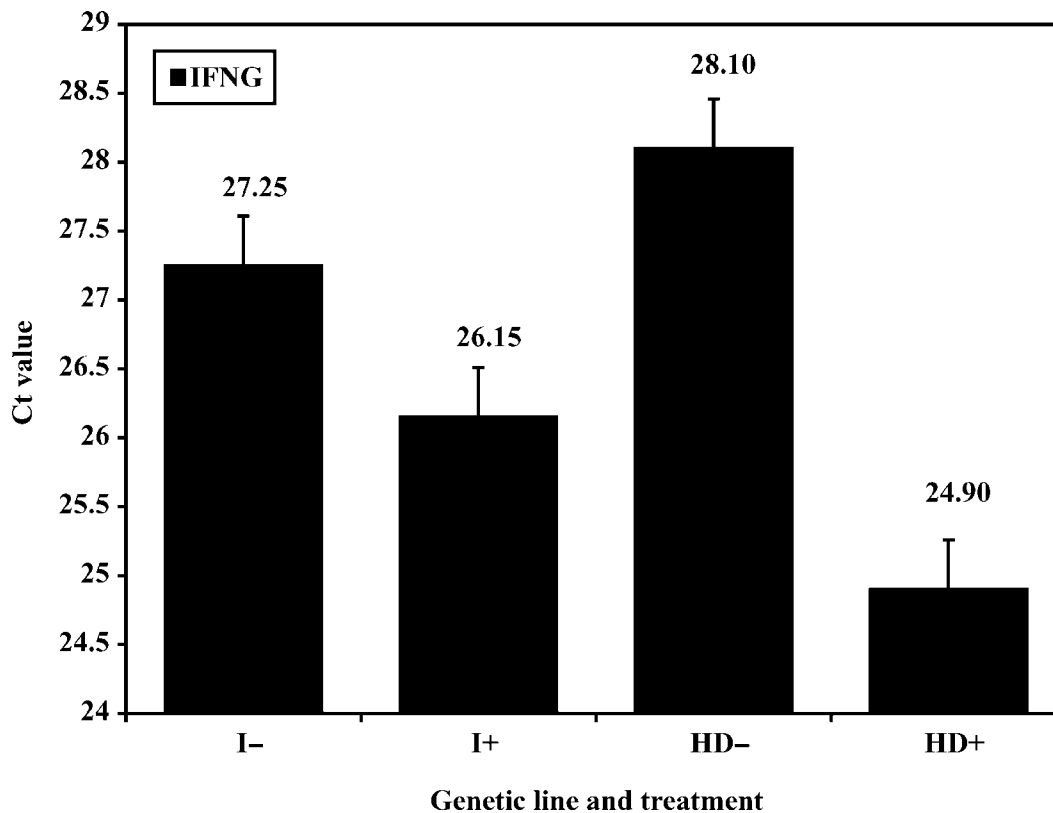
Least squares means for cytokine protein levels are listed in Table 5. Sera were tested from all 56 pigs at 14 dpi for each cytokine. Standard curves associated with cytokines for IL10 from BioSource and IL12/IL23p40 and TNF- $\alpha$  from R&D Systems were not sensitive enough to determine their low protein concentration in the serum. Data for these cytokines are not reported.

**IFNG.** Interactions among effects in the model existed ( $P < 0.0001$ ) for levels of IFNG (Table 3) and are

illustrated in Figure 9. Serum of all pigs had minimal amounts of IFNG before infection. The low-class pigs had increased levels of IFNG (low responding I pigs =  $26.43$  and LHD+ =  $31.02$  pg/mL) at 4 dpi. Levels were still elevated in high- and low-class pigs 7 dpi. The HD pigs produced substantially greater amounts of serum IFNG protein than I pigs, especially 7 dpi and in the high class. Levels of IFNG 14 dpi were lower than at 7 dpi, except for the I pigs with high response to PRRSV infection for which levels continued to increase over time.

**IL1B and IL6.** Interactions were not important for serum levels of IL1B or IL6 (Table 3); however, levels differed ( $P < 0.01$ ) between lines for both cytokines. Day did not affect the level of these cytokines. Levels of IL1B were greater for HD pigs than I pigs ( $636.5 \pm 256.0$  pg/mL); however, levels did not differ between high-class and low-class pigs. Similarly, HD pigs had  $670.3 \pm 265.0$  pg/mL greater concentration of IL6 than I pigs.

**IL8.** Interactions of day  $\times$  class and day  $\times$  treatment for serum levels of IL8 existed (Table 3) and are illustrated in Figure 10. Pigs in the low class, regardless of whether they were infected with virus, had  $339.4 \pm 113.9$  pg/mL greater levels of IL8 before infection and  $292.6 \pm 134.9$  pg/mL greater levels 4 dpi than high-class pigs. However, the amount of IL8 protein in high-



**Figure 5.** Interferon gamma (IFNG) expression level (cycle threshold value; Ct) in lung (SEM = 0.36) tissue of pigs of the NE Index line (I) or Hampshire-Duroc (HD) crossbreds that were uninfected (–) or infected (+) with PRRSV (line × treatment,  $P < 0.01$ ).

class pigs was  $229.5 \pm 158.5$  pg/mL greater 7 dpi and  $626.14 \pm 130.34$  pg/mL greater 14 dpi than in low-class pigs. Infected pigs had greater levels of IL8 postinfection than their uninfected littermates. Pigs in the low class had an initial increase at 4 dpi, but levels returned to normal by 7 dpi. The high-class pigs had a continual elevation to 14 dpi, indicating they were still responding to the virus.

### Correlations

Correlations among gene expression patterns in the lung ranged from  $-0.09$  to  $0.88$  (Table 6). Correlations among expressions of innate genes were positive, ranging from  $0.10$  between expression of INFA and IL6 to  $0.84$  between IL1B and IFNA. Moderate correlations between expression of IL8 and IL1B ( $0.66$ ) and between IL6 and CSF2 ( $0.69$ ) existed. Correlations among expressions of Th1 genes were positive, ranging from  $0.60$  between IL8 and IL12B to  $0.81$  between TNF- $\alpha$  and STAT1. Correlations of expression of IL10, a T-regulatory gene, with expression of other genes were positive, but were greater with the Th1 genes ( $0.63$  to  $0.88$ ) than the innate genes ( $0.21$  to  $0.62$ ).

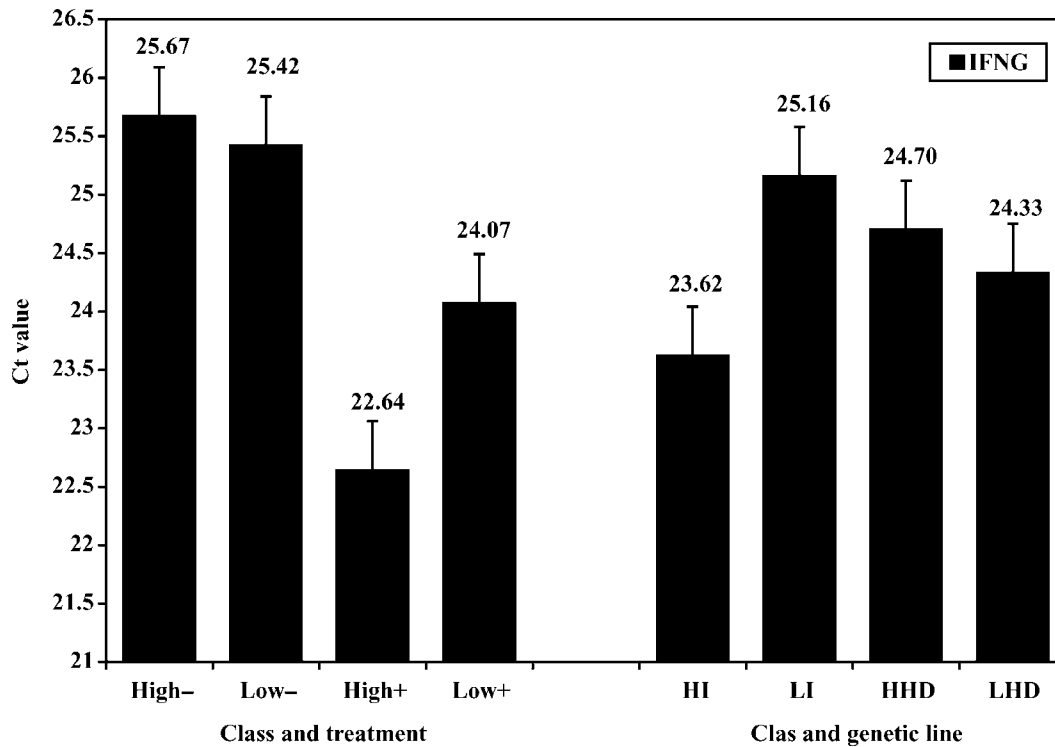
Correlations among gene expression values in the BLN ranged from  $-0.20$  to  $0.77$  (Table 7). Expression of IFNA was negatively correlated with expressions of

IL1B, IL8, and IL6 ( $-0.06$ ,  $-0.02$ , and  $-0.20$ , respectively); however, these associations were very weak indicating that expressions of these genes in BLN are essentially uncorrelated. A moderate correlation of  $0.69$  existed between expression of IL1B and IL8. Correlations among the Th1 genes were positive ranging from  $0.16$  to  $0.77$ . Correlations of expression of IL10 with genes other than INFA were positive, but were more consistent with the Th1 genes ( $0.49$  to  $0.66$ ) than the innate genes ( $-0.10$  to  $0.69$ ).

Correlations among gene expression values of the same gene in lung and BLN ranged from  $0.01$  to  $0.40$  (Table 8, diagonal values). Correlations of expression of genes in lung with other genes in BLN (Table 8, off diagonal elements) tended to be positive, but low, ranging from  $-0.17$  to  $0.36$ . With the exception of the correlation of  $0.92$  between cytokine protein levels of IL1B and IL6, correlations among cytokine proteins of the various genes were negative and not significant (Table 9).

### DISCUSSION

Mean phenotypic responses (Petry et al., 2005) indicated that the HD pigs were more susceptible to PRRSV than I pigs. Infected HD pigs gained less weight from 0 to 14 dpi; had greater rectal temperatures; greater



**Figure 6.** Interferon gamma (IFNG) expression level (cycle threshold value; Ct) in BLN (SEM = 0.42) tissue for pigs classified as having high or low disease burden by the principal component analysis and that were uninfected (–) or infected (+) with PRRSV or were of the NE Index line (I) or Hampshire-Duroc (HD) crossbreeds (class × treatment and class × line,  $P < 0.05$ ).

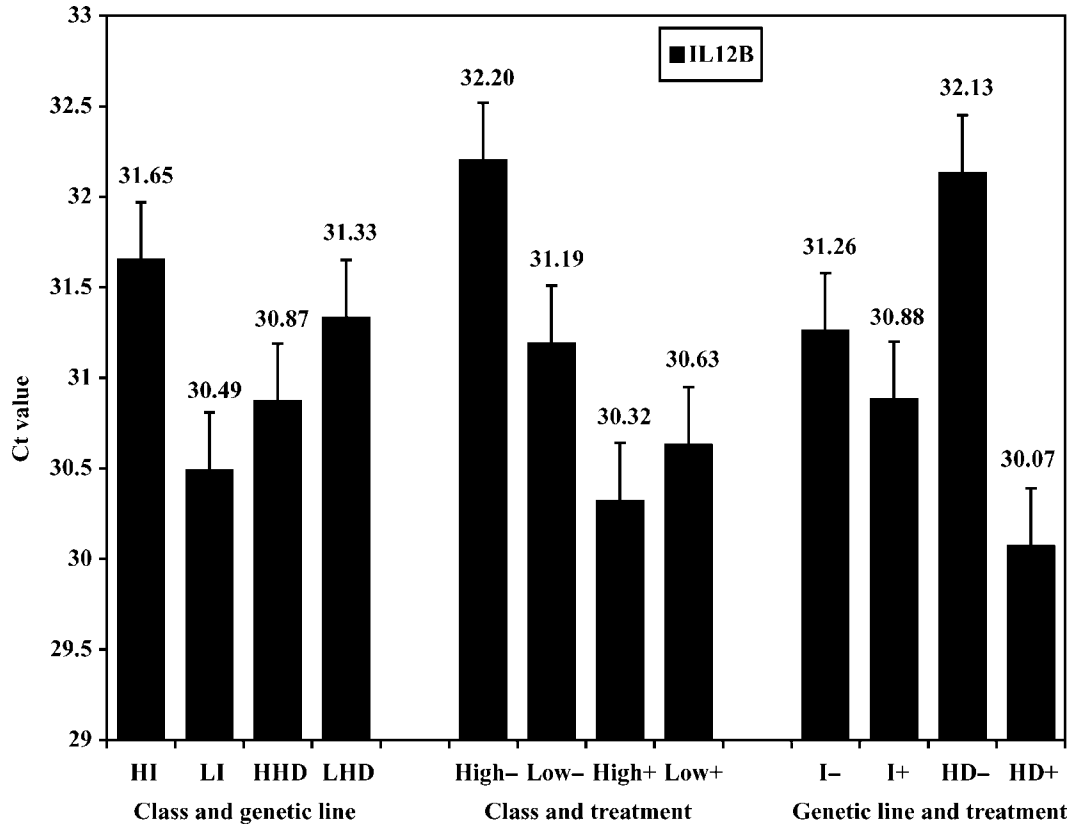
viremia in serum, lung and BLN; and had greater incidence of lung lesions (Table 2). Infection with PRRSV significantly affected expression of every gene evaluated except IL15 in lung or BLN. In all cases except expression of IFNA and CSFT in BLN, infection caused greater expression of genes. Therefore, the general tendency was that infection with PRRSV increased expression of the limited number of immune function genes evaluated.

The first PC of all variables measured was used to describe responses of pigs to PRRSV infection. Pigs in the right tail (high class) of the PC distribution were considered more susceptible. They replicated the virus at very high rates in serum, lung, and BLN (as high as  $10^{5.5}$  CCID<sub>50</sub>/mL), had minimal or negative weight change, high sample to positive ELISA S/P ratios, and greater incidence of lung lesions. Pigs in the other tail (L class), considered to be more resistant, had low viral replication rates in serum, lung, and BLN, as low as  $10^{0.7}$  CCID<sub>50</sub>/mL, positive weight change, low or no S/P ratios, and low or no incidence of lung lesions.

Averaged across infected and uninfected pigs, expression of only CSF2 in BLN differed ( $P < 0.01$ ) between I and HD pigs, and interaction of line × class (high vs. low responders) was significant only for expressions of IL12B in lung ( $P < 0.01$ ) and IFNG and IL6 in BLN ( $P < 0.05$ ). Interactions of line × treatment (infected vs.

uninfected) existed for expression of IFNG, IL12B, IL8, and TNF- $\alpha$  in lung and IFNA in BLN. There was a greater difference between infected and uninfected HD than in I pigs for expressions in lung of IFNG (Figure 5), IL12B (Figure 7), and IL8 (Figure 4). Expression of TNF- $\alpha$  in lung was similar for infected and uninfected I-pigs (28.04 vs. 27.93), but greater for infected than uninfected HD pigs (27.31 vs. 28.78 Ct). The only different pattern existed for expression of IFNA in BLN for which there was little difference between infected and uninfected I pigs (30.27 vs. 30.25 Ct), but infected HD pigs had less expression than uninfected HD pigs (30.48 vs. 29.34 Ct).

One objective was to determine whether response to infection was a general characteristic of high- and low-class pigs or whether expression differences could be measured only in the presence of the virus. From a genetic selection standpoint, it would be desirable to select on a trait in uninfected pigs that is correlated with a response in infected pigs. Thus, expression of genes in uninfected littermates to high and low class pigs was evaluated. Expression in lung of 3 genes, IFNG, STAT1, and IL10, was greater ( $P < 0.05$ ) in high than low pigs, and there was no class × treatment interaction (Table 3), indicating that the difference was similar in pigs infected with PRRSV and their uninfected littermates. Of the genes studied, these are candidates



**Figure 7.** Interleukin 12 beta (IL12B) expression levels (cycle threshold value; Ct) in lung (SEM = 0.32) tissue for pigs classified as having high or low disease burden by the principal component analysis and that were uninfected (-) or infected (+) with PRRSV or were of the NE Index line (I) or Hampshire-Duroc (HD) crossbreds (class  $\times$  line, class  $\times$  treatment, and line  $\times$  treatment,  $P < 0.01$ ).

for additional research to determine whether their expression in uninfected pigs may be useful in selecting for resistance to PRRSV. Mean Ct values for high and low class pigs were 25.67 vs. 27.54 for INFG, 20.17 vs. 21.40 for STAT1, and 25.04 vs. 27.18 for IL10.

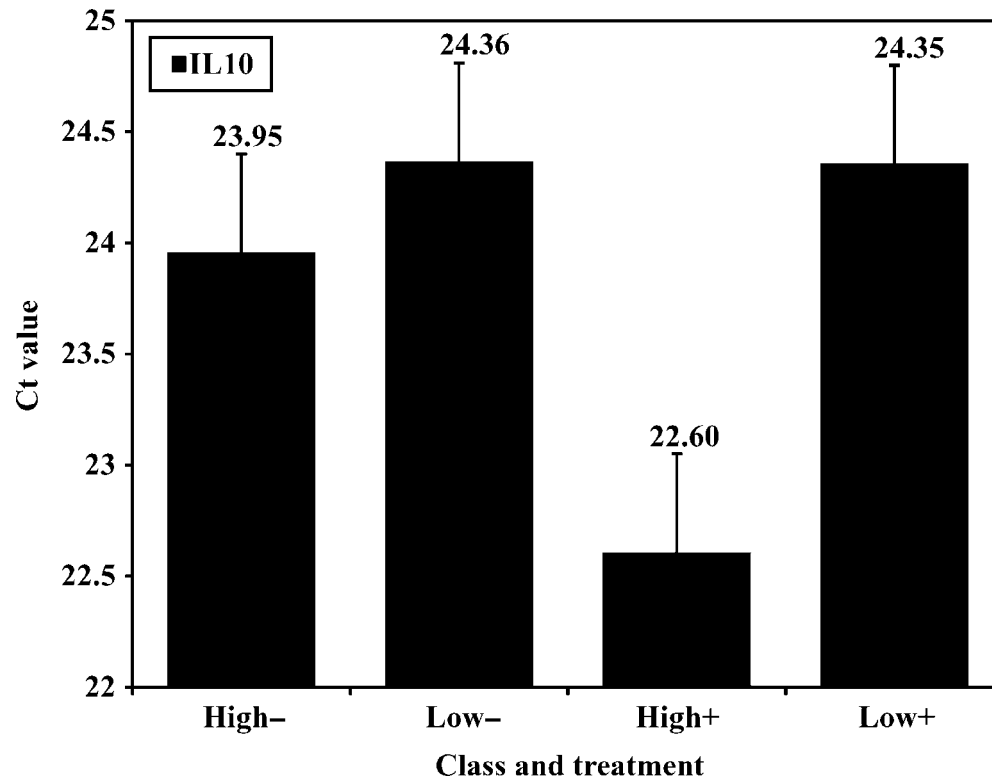
Interaction of class  $\times$  treatment existed in expression of several other genes. For these genes, there was little difference between uninfected high and low pigs and a significant difference between infected high and low pigs (IL1B, Figure 3; IL8, Figure 4; INFG, Figure 6) or the pattern was inconsistent (IL12B, Figure 7). Therefore, expression of these genes in uninfected pigs will not likely be useful predictors of response to infection.

Genetic variation for disease resistance is due to effects of genes that regulate innate and adaptive immune responses. Typically, for viruses Th1 IFNG dominates the response (Murtaugh et al., 2002). Variation in gene expression and cytokine protein levels among pigs and differences between lung and lymph tissue does not in itself indicate genetic variation. However, line differences and interactions among line, treatment, and class found for several genes are evidence that genetic variation in the mechanisms involved in immune responses to PRRSV exists. In general, high-class pigs had greater expression of the innate genes studied

(IFNA, IL1B, IL6, IL8, and CSF2) at 14 dpi in response to PRRSV than low-class pigs. Infected pigs usually had greater levels of expression of innate genes than uninfected pigs, but not in all instances.

Labarque et al. (2003) demonstrated that the ultimate outcome of the interaction between PRRSV and the pig is determined by the pig's response, which is highly variable. Clinical outcomes were inconsistent when naive or immune pigs were infected with PRRSV. Variation is likely due to the variability within pig populations in their innate and adaptive immune responses (Royae et al., 2004; Xiao et al., 2004). However, Thanawongnuwech et al. (2004) found that pigs infected with both PRRSV and *Mycoplasma hyopneumoniae* had greater percentage of increased clinical disease and slower viral clearance than pigs infected with either pathogen alone. Pigs infected with both PRRSV and *M. hyopneumoniae* also had significantly increased levels of mRNA for many proinflammatory cytokines in pulmonary alveolar macrophages than uninfected control pigs, demonstrating that the response to PRRSV also depends on presence of other pathogens.

These findings raise the possibility that other pathogens may have contributed to the phenotypic responses observed in our study between pigs in the outermost



**Figure 8.** Interleukin 10 (IL10) expression levels (cycle threshold value; Ct) in BLN (SEM = 0.45) tissue for pigs classified as having high or low disease burden by the principal component analysis and that were uninfected (-) or infected (+) with PRRSV (class  $\times$  treatment,  $P < 0.01$ ).

tails of the PC distribution. However, pigs of each line were raised as a cohort before the infection experiment and pigs classified as high and low responders to

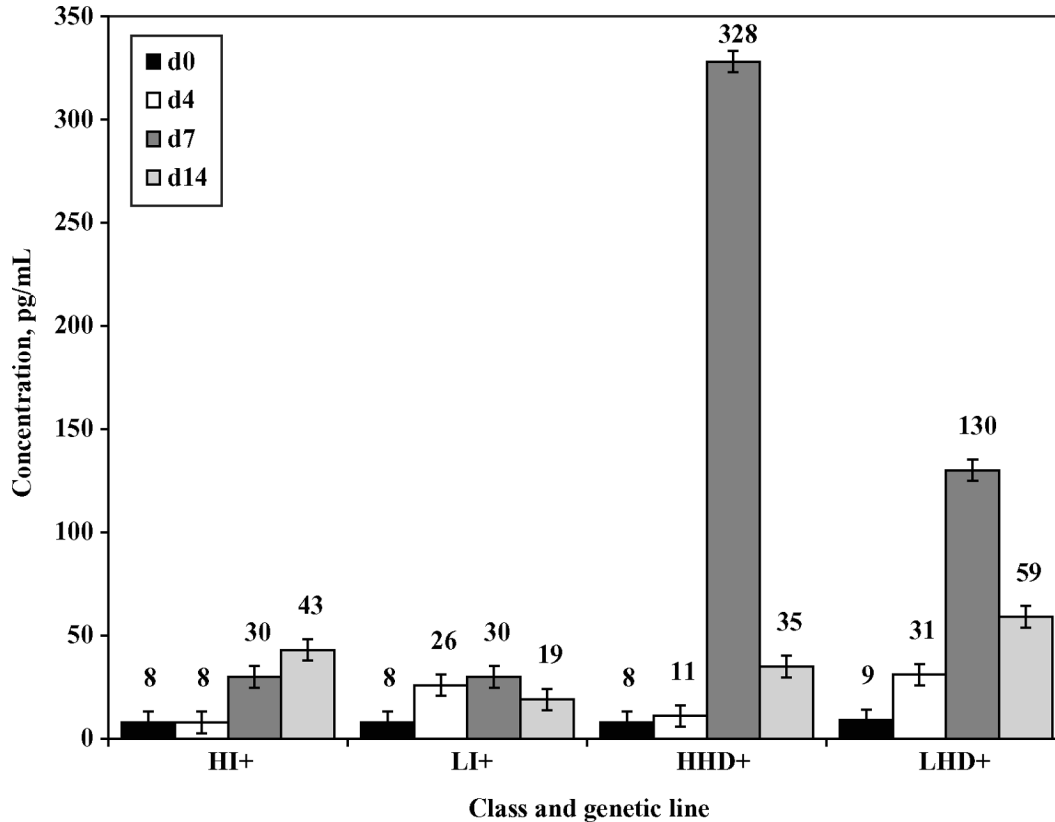
PRRSV were in the same pens during the infection experiment, as were their uninfected littermates. Therefore, before the experiment, pigs of each line were

**Table 5.** Least squares means for concentrations (pg/mL) for interferon gamma (IFNG), IL 1 beta (IL1B), IL8, and IL6 in serum 14 d postinfection (dpi)<sup>1</sup>

Item	Group <sup>2</sup>							
	HHI-	LLI-	HHI+	LLI+	HHHD-	LLHD-	HHHD+	LLHD+
IFNG 0 dpi	8.30	8.00	7.61	8.00	8.14	7.84	8.00	8.82
IFNG 4 dpi	8.00	8.00	7.92	26.43	8.00	8.00	11.00	31.02
IFNG 7 dpi	82.30	8.00	30.41	30.14	8.14	7.84	328.43	130.42
IFNG 14 dpi	16.57	34.14	42.86	19.43	16.72	14.57	35.14	58.57
IL1B 0 dpi	311.37	30.00	203.37	30.00	1,026.44	399.83	1,116.88	205.14
IL1B 4 dpi	105.86	30.00	98.37	30.00	954.00	597.29	945.00	520.14
IL1B 7 dpi	30.00	30.00	37.42	30.00	19.44	399.83	1,698.71	526.94
IL1B 14 dpi	319.43	25.00	367.86	25.00	872.29	672.00	920.29	598.43
IL8 0 dpi	648.21	988.71	509.22	662.57	867.28	1,108.34	374.86	997.60
IL8 4 dpi	981.71	800.57	1,016.99	1,253.71	729.57	1,272.86	1,162.57	1,734.20
IL8 7 dpi	970.21	558.14	916.76	604.14	840.28	823.34	1,217.57	1,041.20
IL8 14 dpi	971.71	522.14	1,145.86	471.57	949.86	613.14	1,678.00	634.00
IL6 14 dpi	243.86	40.00	102.86	62.29	944.29	679.43	873.43	632.86

<sup>1</sup>Serum collected from infected and control pigs was assayed for cytokine protein levels using the commercial tests noted in Materials and Methods.

<sup>2</sup>HHI- = High viremia, high symptoms, uninfected NE Index line (I) pig; LLI- = Low viremia, low symptoms, uninfected NE Index line (I) pig; HHI+ = High viremia, high symptoms, infected NE Index line (I) pig; LLI+ = Low viremia, low symptoms, infected NE Index line (I) pig; HHHD- = High viremia, high symptoms, uninfected Hampshire-Duroc (HD) crossbred pig; LLHD- = Low viremia, low symptoms, uninfected Hampshire-Duroc (HD) crossbred pig; HHHD+ = High viremia, high symptoms, infected Hampshire-Duroc (HD) crossbred pig; and LLHD+ = Low viremia, low symptoms, infected Hampshire-Duroc (HD) crossbred pig.



**Figure 9.** Interferon gamma (IFNG) cytokine protein levels (pg/mL) in serum (SEM = 5.21) at d 0, 4, 7, and 14 postinfection for pigs classified as having high or low disease burden by the principal component analysis and that were uninfected (–) or infected (+) with PRRSV and that were of the NE Index line (I) or Hampshire-Duroc (HD) crossbreds (day  $\times$  class  $\times$  line  $\times$  treatment,  $P < 0.01$ ).

expected to have been exposed to the same pathogens, and during the experiment, pigs within a room, which contained pigs of each line, were expected to have been exposed to the same pathogens. Furthermore, there was little evidence of pneumonia due to *M. hyopneumoniae* in these pigs (Petry et al., 2005). Therefore, differences between pigs in the tails of the distribution are most likely to be due to differential responses to PRRSV and not to other pathogens.

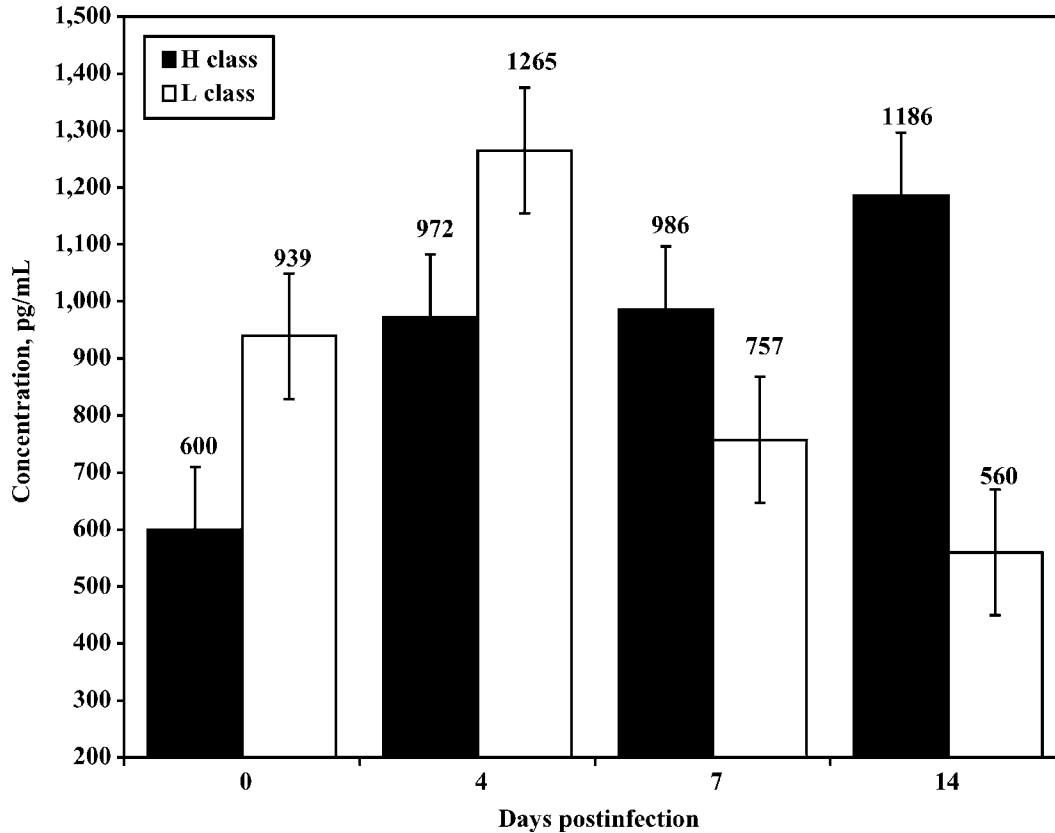
Breed differences seem to play a role in determining resistant/susceptibility of pigs to PRRSV. Halbur et al. (1998) found that Duroc pigs that were infected with PRRSV had greater ELISA S/P ratios, lower ADG, and increased severity of PRRSV-induced lesions in the lung than Meishan pigs. In an infection experiment similar to ours, Vincent et al. (2005, 2006) compared pigs from 2 lines to a challenge with PRRSV at 6 wk of age. They used an in vitro fluorescence-activated cell sorting (FACS) assay to determine the percentage of PRRSV-infected macrophages. A line derived from the Large White pigs was characterized as FACS<sup>hi</sup>, whereas a line derived from Duroc and Pietrain pigs was characterized as FACS<sup>lo</sup>. The FACS<sup>lo</sup> line had more severe clinical disease 10 dpi, although differences between lines diminished by 21 dpi. A consistent result of these

studies and ours is that pigs from lines or breeds with high reproduction (Meishan, Large White, and NE Index line) were more resistant to the effects of the virus than pigs from lines selected for lean growth rate (Duroc, Pietrain, and HD). This finding could be coincidence but may be related to the stage of growth in which responses to virus were measured, which was in the growing pig. Because PRRSV is specific to the respiratory tract, infection of the lungs may have greater negative effects on pigs with greater rates of lean growth. Reiner et al. (2002) found similar trends for pseudorabies virus infected pigs.

With a sow-model, Lowe et al. (2005) found that genetics may affect the rate of PRRSV-induced abortions, possibly due to levels of circulating IFNG secreting cells. Our data indicate that early postinfection (4 dpi) increases in serum IFNG may be associated with resistance. Very high serum IFNG, however, may not be protective because greater IFNG levels were found in HD pigs as compared with I pigs, and the greatest serum PRRSV burden was in the HHD pigs (Figure 9).

Genetic variation in pigs in response to pathogens or to modulation of the immune system has been demonstrated (Mallard et al., 1998; Wilkie and Mallard, 1999).





**Figure 10.** Interleukin 8 (IL8) cytokine protein levels (pg/mL) in serum (SEM = 110.50) at d 0, 4, 7, and 14 postinfection with PRRSV for pigs classified as having high or low disease burden by the principal component analysis (class  $\times$  day,  $P < 0.01$ ).

After 8 generations of selection for antibody and cell-mediated immune responses, high, low, and control lines had diverged for growth rate, antibody response to various antigens, and response to *Mycoplasma hyorhinis*. Wilkie and Mallard (1999) concluded that genetic variation in response to certain antigens and to *M. hyorhinis* exists. Though pathways and mechanisms in-

involved in resistance were not characterized, it was concluded that the genetic variation was polygenic, regulating both innate resistance and acquired immunity. Studies by Galina-Pantoja et al. (2006) found that the proportion of several peripheral cell subsets appeared to predict growth during the entire productive life of the pig.

**Table 6.** Correlations<sup>1</sup> among gene expression (cycle threshold value) patterns in the lung

Trait <sup>2</sup>	IFNA	IL15	RPL32	STAT1	IL1B	IL12B	CSF2	IL8	IL10	IL6	TNF- $\alpha$
IFNG	0.20	0.60	0.46	0.80	0.15	0.64	0.51	0.48	0.73	0.51	0.66
IFNA		-0.07	0.09	0.15	0.84	0.38	0.11	0.53	0.21	0.10	0.46
IL15			0.78	0.77	-0.09	0.66	0.74	0.42	0.68	0.66	0.62
RPL32				0.77	0.08	0.66	0.76	0.48	0.69	0.83	0.68
STAT1					0.18	0.73	0.70	0.61	0.88	0.73	0.81
IL1B						0.28	0.12	0.66	0.21	0.17	0.44
IL12B							0.65	0.60	0.63	0.64	0.69
CSF2								0.51	0.57	0.69	0.69
IL8									0.55	0.53	0.71
IL10										0.62	0.79
IL6											0.63

<sup>1</sup>Correlations  $> 0.28$  have a  $P < 0.05$  and correlations  $> 0.35$  have a  $P < 0.01$ .

<sup>2</sup>IFNG = interferon gamma; IFNA = interferon alpha; IL15 = IL 15; RPL32 = ribosomal protein L32; STAT1 = signal transducer and activator of transcription 1-alpha; IL1B = IL 1 beta; IL12B = IL 12 beta; CSF2 = colony stimulating factor 2; and TNF- $\alpha$  = tumor necrosis factor alpha.

**Table 7.** Correlations<sup>1</sup> among gene expression (cycle threshold value) patterns in the bronchial lymph node

Item	Trait <sup>2</sup>										
	IFNA	IL15	RPL32	STAT1	IL1B	IL12B	CSF2	IL8	IL10	IL6	TNF- $\alpha$
IFNG	-0.02	0.60	0.68	0.77	0.26	0.39	0.23	0.48	0.63	0.58	0.58
IFNA		-0.03	0.02	0.00	-0.06	-0.02	0.03	-0.02	-0.10	-0.20	0.00
IL15			0.70	0.68	0.32	0.16	0.40	0.31	0.60	0.44	0.37
RPL32				0.74	0.35	0.41	0.49	0.38	0.60	0.62	0.61
STAT1					0.37	0.28	0.35	0.39	0.65	0.63	0.47
IL1B						0.43	0.42	0.69	0.60	0.51	0.50
IL12B							0.39	0.26	0.49	0.64	0.70
CSF2								0.24	0.58	0.54	0.49
IL8									0.59	0.34	0.39
IL10										0.69	0.66
IL6											0.68

<sup>1</sup>Correlations > 0.28 have  $P < 0.05$  and correlations > 0.35 have  $P < 0.01$ .

<sup>2</sup>IFNG = interferon gamma; IFNA = interferon alpha; IL15 = IL 15; RPL32 = ribosomal protein L32; STAT1 = signal transducer and activator of transcription 1-alpha; IL1B = IL 1 beta; IL12B = IL 12 beta; CSF2 = colony stimulating factor 2; and TNF- $\alpha$  = tumor necrosis factor alpha.

Correlations in gene expression patterns between tissues were weak, indicating that patterns for 1 tissue cannot be extrapolated to other tissues. This is expected for local mucosal responses, as reported previously by Dawson et al. (2005) for different parasitic infections in pigs. The earliest detection of IFNG was in the liver and lymph node; however, expression patterns of upstream regulatory factors controlling IFNG expression were assessed and found to be upregulated at early stages of infection, but less upregulated at later stages of the infection. This further illustrates the critical role timing of the pathways plays in immune responses.

Correlations among expressions of innate genes within each tissue were moderate to high and positive, indicating that these genes respond similarly to the same stimuli or are controlled by similar genetic path-

ways. Also, correlations among the expressions of Th1 genes were moderate to high and positive, indicating that these genes also respond similarly to PRRSV when measured 14 dpi. Expression of IL10, which is a T-regulatory gene, was highly correlated with expression of the Th1 genes (0.63 to 0.88) indicating that IL10, which suppresses macrophages and dendritic cells, and the Th1 genes are responding similarly to early lung and BLN infection with PRRSV. There was a strong association between cytokine levels of IL1B and IL6 (0.92). These genes are both part of the innate immune system and have overlapping functions.

Pigs classified as low responders to PRRSV had  $339.4 \pm 113.9$  pg/mL greater levels (57% more) of IL8 prior to infection than high-class pigs, whereas at 14 dpi high-class pigs had  $626.1 \pm 130.3$  pg/mL greater levels

**Table 8.** Correlations<sup>1</sup> among gene expression (cycle threshold value) patterns across the lung and bronchial lymph node

Item	Trait <sup>2</sup>											
	IFNG	IFNA	IL15	RPL32	STAT1	IL1B	IL12B	CSF2	IL8	IL10	IL6	TNF- $\alpha$
IFNG	0.40	0.11	0.17	0.04	0.30	0.06	0.17	0.06	0.04	0.28	0.08	0.13
IFNA	0.00	0.15	-0.06	-0.05	0.06	0.20	-0.06	-0.06	0.24	-0.07	-0.07	0.00
IL15	0.32	-0.10	0.23	0.22	0.34	-0.17	0.06	0.19	0.05	0.35	0.26	0.27
RPL32	0.16	-0.01	0.16	0.12	0.20	-0.06	-0.01	0.12	0.01	0.18	0.09	0.11
STAT1	0.30	0.20	0.06	0.00	0.34	0.19	0.06	0.08	0.13	0.36	0.05	0.27
IL1B	-0.01	0.07	0.04	0.11	0.10	0.14	0.05	-0.04	0.16	0.20	0.14	0.11
IL12B	0.07	0.23	0.19	0.20	0.14	0.12	0.32	0.02	0.14	0.16	0.14	0.15
CSF2	-0.13	0.05	0.03	0.21	0.03	-0.08	0.02	0.09	0.03	0.09	0.09	0.08
IL8	0.12	0.01	-0.01	-0.06	0.11	0.02	-0.07	-0.15	0.01	0.23	-0.05	0.00
IL10	0.16	-0.04	0.01	0.03	0.16	-0.16	0.00	-0.11	-0.06	0.27	0.03	0.02
IL6	0.22	0.13	0.18	0.24	0.29	0.07	0.16	0.05	0.09	0.36	0.16	0.28
TNF- $\alpha$	0.14	0.09	0.14	0.15	0.19	0.03	0.15	0.08	0.10	0.14	0.07	0.17

<sup>1</sup>Correlations > 0.28 have  $P < 0.05$  and correlations > 0.35 have  $P < 0.01$ .

<sup>2</sup>IFNG = interferon gamma; IFNA = interferon alpha; IL15 = IL 15; RPL32 = ribosomal protein L32; STAT1 = signal transducer and activator of transcription 1-alpha; IL1B = IL 1 beta; IL12B = IL 12 beta; CSF2 = colony stimulating factor 2; and TNF- $\alpha$  = tumor necrosis factor alpha.

**Table 9.** Correlations<sup>1</sup> among cytokine protein levels, pg/mL

Trait <sup>2</sup>	IL1B	IL8	IL6
IFNG	0.00	-0.14	-0.03
IL1B		-0.04	0.92
IL8			-0.15

<sup>1</sup>Correlations > 0.28 have  $P < 0.05$  and correlations > 0.35 have  $P < 0.01$ .

<sup>2</sup>IL1B = IL 1 beta; and IFNG = interferon gamma.

of IL8 than low-class pigs. The IL8 cytokine is involved in neutrophil chemotaxis, which are phagocytic cells that play a major role in defense of a host against infection. Possibly, pigs classified as low responders had a quick, strong response to PRRSV naturally because more IL8 was circulating in the blood, providing innate protection to shed the virus quickly. No supporting data for this finding were found; thus, this finding needs further investigation to determine whether IL8 in serum of uninfected pigs may be a predictor of response to PRRSV infection. The IL8 cytokines are relatively easily measured and could be easily included in a selection program without PRRSV infection.

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