Original Article

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Association between endotoxin levels in dust from indoor swine housing environments and the immune responses of pigs

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Indoor animal husbandry environments are inevitably contaminated with endotoxins. Endotoxin exposure is associated with various inflammatory illnesses in animals. This cross-sectional study evaluated the relationship between the degree of endotoxin exposure and the cellular and humoral immune profiles of fattening pigs. Blood samples were taken from the jugular vein of 47 pigs from ten pig farms in Korea. Whole blood cell counts and plasma immunoglobulin (Ig) classes were determined. Peripheral-blood mononuclear cells were stimulated *in vitro* with concanavalin A for 48 h, and cytokines released into culture supernatants were measured. The barns in which the pigs lived were assessed for endotoxin levels in the total and respirable dust by using the limulus amebocyte lysate kinetic QCL method. Low and high endotoxin exposures were defined as $\leq$ 30 and $>$ 30 EU/m^3, respectively. Compared to pigs with low endotoxin exposure (n = 19), highly exposed pigs (n = 28) had higher circulating neutrophil and lymphocyte (particularly B cells) counts, IgG and IgE levels, interferon-gamma (IFNγ) and interleukin (IL)-4 productions, and lower IgA levels and tumor necrosis factor-alpha (TNFα) production. The IL-4, IFNγ, and TNFα levels significantly correlated with endotoxin level and/or pig age. Constant exposure of pigs to high levels of airborne endotoxins can lead to aberrant immune profiles.

Keywords: cellular immunity, endotoxins, organic dust, swine

Introduction

Due to the presence of feeding materials and feces, the environment inside swine facilities is very dusty [7,30]. A study on Korean swine farms reported that 60% to 70% of the total dust in such facilities comes from feeding materials, which rises to approximately 90% during feeding time [19]. The dust is a biologically active aerosol because it contains microorganisms such as bacteria, viruses, and fungi and their organic compounds, including endotoxins [22].

Endotoxins are one of the most ubiquitous organic compounds in agricultural settings. This is partly due to their heat resistance, which allows them to persist in the environment even under extreme conditions [21]. Airborne endotoxin levels can be estimated by using the limulus amebocyte lysate (LAL) assay, which measures endotoxin levels as functional (bioactive) endotoxin units (EUs) per cubic meter of air (EU/m^3) [33,35]. There is still no internationally accepted endotoxin exposure threshold that indicates safe levels of exposure, even in humans. Several studies have proposed that the following thresholds may be useful for humans: a no-observed-adverse-effect level of 17 EU/m^3 [25] or $<$ 100 EU/m^3 [20], and a lowest observed effect level of 30 to 75 EU/m^3 [20]. However, Park *et al*. [28] suggested that indoor airborne endotoxin levels in homes as low as 0.02 to 19.8 EU/m^3 could have a role in sick-building syndrome.

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An endotoxin is a potent immunogenic stimulant, and even small amounts can non-specifically stimulate the immune system [21,28,38]. Our previous study showed that swine-farm workers who were exposed to high endotoxin levels had type 2-helper (T_H2)-skewed immune profiles; their peripheral-blood mononuclear cells (PBMCs) stimulated with phorbol 12-myristate 13-acetate and ionomycin produced lower levels of the proinflammatory cytokines interferon-gamma (IFNγ) and tumor necrosis factor-alpha (TNFα) and higher levels of the anti-inflammatory cytokines interleukin (IL)-4 and IL-13 than a control group of office workers. This suggests that endotoxin exposure may promote allergic responses [16]. Similarly, when healthy human subjects [1] and mice [40] inhaled low endotoxin doses, they exhibited T_H2-skewed immune profiles. While the effect of endotoxin exposure on the immune status of livestock animals has not yet been extensively investigated, Kullik *et al*. [18] showed that intravenous injection of pigs with lipopolysaccharide (LPS) significantly increased their plasma TNFα and IL-6 levels. In addition, when normal porcine monocytes are differentiated into macrophages in the presence of organic dust extract from swine husbandry facilities, their differentiation and responses to LPS are impaired [29]. Similarly, pig barn dust extract treatment *in vitro* negatively affects porcine macrophage function [17].

Recently, we reported that indoor dust endotoxin levels in swine-confinement facilities do not associate significantly with the isolation of Gram-negative bacteria from the air in these buildings [33]. This suggests that when assessing the effect of endotoxins on the health of animals or workers, indoor endotoxin levels may be a more useful measure of endotoxin exposure than isolation of Gram-negative bacteria. Hence, to test the hypothesis that exposure to endotoxins in natural husbandry settings can alter the immune responses of pigs, we measured endotoxin levels in several swine farms and assessed the relationship of endotoxin level with the immunological profiles of the swine in these farms.

Materials and Methods

Pig farms and blood collection

Ten pig farms in six counties in Korea participated in this study. They were selected from a list that was prepared by a swine farmers' cooperative and were included in the study if the farmers allowed us to enter the pig-confinement buildings. The buildings were visited between July and September in 2012, 2014, or 2015. All pig farms employed open-type housing in which ventilation was provided by large purpose-built windows with a curtain winching system. In all farms, the stocking density ranged from 0.8 to 1.7 m^2/pig, which is within the recommended stocking density (0.8 m^2/pig for fattening pigs) of the Korean government [26]. In each farm, three to 10 pigs with no apparent clinical or pathological abnormalities (age ranged from 90 to 150 days) were randomly chosen for blood collection irrespective of sex. Blood samples were collected by local veterinarians. A maximum of 10 mL of blood was drawn aseptically from the jugular vein of each pig and placed into EDTA-coated vacutainers [18]. All animal handling, blood collection, and experimental procedures were approved by the Institutional Animal Care and Use Committee of Daegu Catholic University (IACUC No. CUD IACUC-2012-10).

Dust collection and endotoxin measurement

Several reports show that the dust and endotoxin concentrations are highest in the daytime since this is when the animals are fed and are most active [19]. Therefore, we measured the total indoor dust levels in the pig farms for 8 h from 9 AM until 5 PM. For this, we used polyvinyl chloride (PVC) membrane filters (SKC, USA) with a 2-stage cassette at a flow rate of 2.0 L/min. We also measured the concentration of respirable dust, particulate matter less than or equal to 10 μm (PM10) by using a PVC membrane filter with a 10 mm Dorr-Oliver nylon cyclone at a flow rate of 1.7 L/min for 8 h [16,34,35]. Both total and respirable dust samples were collected at two different locations in each farm; namely, at one- and two-thirds of the total distance from the entrance gate. Blank and sample filters were weighed at least three times by using an electronic micro-balance (Quintix 125D; Sartorius, Germany).

Endotoxin concentrations in dust were measured as described previously [32,33,35]. Briefly, endotoxin was extracted from the filters by adding 3 mL of endotoxin-free LAL water (Lonza, USA) with 0.5% Tween 20 followed by shaking for 1 h at 350 r/min. The endotoxin concentrations in the collected supernatants were measured by using a LAL Kinetic QCL kit (Lonza) according to the manufacturers' instructions. Given the proposed threshold endotoxin levels described in the Introduction, we decided that 30 EU/m^3 in total dust would serve as the threshold distinguishing high and low endotoxin levels in swine farms. Consequently, the pig farms with $\leq$ 30 and $>$ 30 EU/m^3 in total dust were considered to be low and high endotoxin exposure farms, respectively.

Determination of porcine hematological values

Total and differential white blood cell (WBC), red blood cell (RBC), and platelet counts of each pig were determined by an ADVIA 2120 (Siemens, Germany) automated hematology analyzer. Veterinary software adapted for pigs was used to analyze the data.

PBMC collection and lymphocyte phenotyping

PBMCs were isolated by Ficoll-Hypaque density-gradient centrifugation (Ficoll-Paque Plus; GE Healthcare Life Sciences, USA) [16,34,35]. Lymphocyte subpopulations were analyzed by using three-color flow cytometry (FACScan; BD, USA). Porcine B and T cells were identified by using mouse anti-pig

CD1-FITC Ab and mouse anti-pig CD3ε antibodies (Southern Biotech, USA), respectively. Porcine cytotoxic T cells, helper T cells, and $CD4^+CD8^+$ T cells were identified by using mouse anti-pig CD8α-FITC Ab and mouse anti-pig CD4α-FITC antibodies (Southern Biotech). R-phycoerythrin- or FITC-conjugated isotype controls served to control for non-specific background fluorescence.

Enzyme-linked immunosorbent assay quantitation of plasma antibodies

Porcine serum IgG and IgA (Bethyl Laboratories, USA) and IgE (Elabscience, USA) levels were measured by using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions. Optical density was measured at 450 nm (Epoch; Bio-Tek, USA).

T-cell activation and measurement of secreted cytokine levels

Porcine PBMCs (10^6 cells/mL) were resuspended in complete RPMI medium (1 mM nonessential amino acids, 1 mM sodium pyruvate, 1% sodium bicarbonate, 2 mM glutamine, 50 M 2-mercaptoethanol, and 10% heat-inactivated fetal bovine serum) and activated with 5 μg concanavalin A and 10 IU recombinant human IL-2 for 48 h at 37°C in a 5% CO_2 incubator. IL-4 secretion into the medium was determined by using a mouse anti-swine IL-4 monoclonal antibody (mAb) (capture antibody), recombinant swine IL-4 (standard), and a biotin-conjugated mouse anti-swine IL-4 mAb (detection antibody) (Invitrogen, USA). IFNγ secretion was determined by using a mouse anti-swine IFNγ mAb (capture antibody) (Invitrogen), recombinant swine IFNγ (standard) (BioSource, USA), and a biotin-conjugated mouse anti-swine IFNγ mAb (detection antibody) (Invitrogen). IL-12/IL-23 p40 and TNFα were quantitated by using Duoset ELISA kits (R&D Systems, USA).

Statistical analyses

All statistical analyses were performed by using SigmaStat 3.5 (Systat Software, USA). Depending on the normality of the data, the high and low endotoxin groups were compared by using Student's *t*-test or the Mann-Whitney rank-sum test. Differences were considered significant when p was < 0.05. Correlations between husbandry and immunological variables were analyzed by determining Pearson's correlation coefficients.

Results

Endotoxin levels in the pig farms

The high (n = 5) and low (n = 5) endotoxin exposure farms had similar general husbandry conditions; they had similar numbers of pigs in the confinement area, stocking densities, average pig ages, and ventilation modes with curtain winching systems (Table 1). However, the high endotoxin exposure farms tended to have higher total dust levels (mean, 0.87 mg/m^3; range, 0.10–1.54 mg/m^3) than that in the low-exposure farms (mean, 0.58 mg/m^3; range, 0.13–1.06 mg/m^3; $p = 0.0536$). They also had significantly higher respirable dust levels (mean, 0.67 mg/m^3; range, 0.28–0.83 mg/m^3) than that in the low-exposure farms (mean, 0.32 mg/m^3; range, 0.01–0.96 mg/m^3; $p = 0.0001$).

The high endotoxin exposure farms had significantly higher endotoxin levels in the total dust (mean, 443.18 EU/m^3; range, 47.1–1,198.8 EU/m^3) than that in the low endotoxin farms (mean, 13.05 EU/m^3; range, 0–29.6 EU/m^3; $p = 0.0003$). In addition, the high endotoxin farms had significantly higher endotoxin concentrations in the respirable dust (mean, 7.22 EU/m^3; range, 0.55–14.48 EU/m^3) than that in the low endotoxin farms (mean, 1.03 EU/m^3; range, 0.01–2.76 EU/m^3; $p = 0.0000$) (Table 1).

Table 1. Differences in swine husbandry conditions and endotoxin levels in total or respirable indoor dust between farms with high- and low-endotoxin exposure

Husbandry condition	High-endotoxin exposure farms	Low-endotoxin exposure farms	*p* value
No. of swine farms	5	5	
No. of pigs*	604 ± 429	362 ± 153	n.s.
Stocking density (m^2/pig)	1.15 ± 0.25	1.23 ± 0.33	n.s.
Pig age (d)	138.8 ± 13.3	118.4 ± 23.4	n.s.
Total dust (mg/m^3)	0.87 ± 0.60	0.58 ± 0.35	0.0536
Respirable dust (mg/m^3)	0.67 ± 0.21	0.32 ± 0.36	0.0001
Endotoxin in total dust (EU/m^3)	443.18 ± 480.56	13.05 ± 12.07	0.0003
Endotoxin in respirable dust (EU/m^3)	7.22 ± 4.86	1.03 ± 1.22	0.0000

Farms were classed as low- or high-endotoxin exposure when their endotoxin levels in total dust were ≤ 30 and > 30 EU/m^3, respectively. Data are presented as mean ± SD. The *p* values were obtained by comparing the low- and high-endotoxin exposure groups by applying Student's *t*-test or the Mann-Whitney *U* test, as appropriate. EU, endotoxin unit; n.s., no significant difference. *The average number of pigs that were reared in each confinement building.

Hematological cell and lymphocyte subpopulation counts in the blood

Blood was collected from 28 pigs on the high endotoxin exposure farms (mean, 5.6 ± 2.6 pigs/farm) and 19 pigs on the low endotoxin exposure farms (mean, 3.8 ± 1.1 pigs/farm). The pigs from the high endotoxin exposure farms had significantly higher total WBC counts than that in the pigs from the low endotoxin farms (p = 0.0024). Since the two groups did not differ significantly in monocyte, eosinophil, and basophil counts, but did differ significantly in neutrophil and lymphocyte counts (p = 0.0187 and 0.0172, respectively), the higher total WBC counts reflect the higher neutrophil and lymphocyte counts in the highly exposed pigs. The pigs with high and low endotoxin exposure did not differ in RBC and platelet counts. The highly exposed pigs tended to have higher $CD1^+$ B-cell counts than that in the pigs with low endotoxin exposure (p = 0.0546) but did not differ in other lymphocyte subsets (Table 2) [9,24].

Cytokine secretion by *in vitro*-stimulated PBMCs and plasma immunoglobulin levels

PBMC secretion of cytokines that have key roles in cell-mediated immunity was examined. The PBMCs from highly exposed pigs secreted significantly more IFNγ and IL-4 than that in the PBMCs from the pigs with low exposure (p = 0.034 and 0.037, respectively) (Fig. 1). Since the IFNγ:IL-4 ratio of non-specifically activated PBMCs can indicate an immune system skewing toward T_H1 or T_H2 immune responses, we calculated the IFNγ:IL-4 ratio by dividing the IFNγ concentration by the IL-4 concentration in the same culture supernatant and then multiplied the result by 10. High endotoxin exposure was associated with high IFNγ:IL-4 ratios (5.8 ± 1.4 *vs*. 3.9 ± 0.9). The two exposure groups did not differ in terms of PBMC production of IL-12/IL-23 p40 and TNFα (p = 0.951 and 0.513, respectively).

The highly endotoxin-exposed pigs tended to have higher plasma IgG and IgE levels than those with low endotoxin exposure, but the differences were without statistical significance (p = 0.213 and 0.574, respectively). However, high-exposure pigs had significantly lower plasma IgA levels than that in low-exposure pigs (p = 0.009) (Fig. 2).

Relationship between swine husbandry conditions and immunological variables

Correlations between endotoxin level and other husbandry factors with swine immunological variables were analyzed. Table 3 presents only the significant results. Pig age correlated positively with *in vitro* IL-4 (r = 0.398) and IFNγ production (r = 0.622) and negatively with platelet levels (r = −0.679). Total dust endotoxin levels correlated positively with total WBC (r = 0.496), neutrophil (r = 0.420), lymphocyte (r = 0.351), and monocyte (r = 0.505) counts in peripheral blood and *in vitro* TNFα (r = 0.706) and IL-12/IL-23 p40 production (r = 0.663). Respirable dust endotoxin levels correlated positively with *in vitro* IL-4 (r = 0.579) and IFNγ production (r = 0.697) and negatively with *in vitro* TNFα (r = −0.744) and IL-12/IL-23 p40 production (r = −0.563). Thus, total dust endotoxin and respirable dust endotoxin levels had opposite effects on TNFα and IL-12/IL-23 p40 production. Respirable dust endotoxin levels significantly correlated with platelet (r = −0.361) and $CD1^+$ B-cell counts (r = 0.555).

Table 2. Immune cell counts in the peripheral blood of pigs from the high- and low-endotoxin exposure farms*

Immune cell phenotype	High-endotoxin exposure farms	Low-endotoxin exposure farms	p value
Total WBCs (10^3/μL)	19.65 ± 0.98	15.12 ± 0.92	0.0024
RBCs (10^6/μL)	7.33 ± 0.18	7.84 ± 0.63	n.s.
Platelets (10^3/μL)	245.46 ± 27.25	286.68 ± 36.04	n.s.
Neutrophils (10^3/μL)	5.97 ± 0.46	4.21 ± 0.55	0.0187
Lymphocytes (10^3/μL)	11.61 ± 0.69	9.20 ± 0.60	0.0172
Monocytes (10^3/μL)	1.01 ± 0.09	0.80 ± 0.08	n.s.
Eosinophils (10^3/μL)	0.76 ± 0.08	0.68 ± 0.10	n.s.
Basophils (10^3/μL)	0.14 ± 0.02	0.12 ± 0.01	n.s.
$CD1^+$ B cells (%)	8.28 ± 0.98	5.80 ± 0.44	0.0546
$CD4^+$ T cells (%)	12.78 ± 0.87	13.30 ± 0.91	n.s.
$CD8^+$ T cells (%)	19.32 ± 0.85	21.82 ± 1.13	n.s.
$CD4^+$ $CD8^+$ T cells (%)	9.64 ± 0.75	9.97 ± 0.57	n.s.

Data are presented as mean ± SE. The p values were obtained by comparing the low- and high-exposure groups by applying Student's t-test or the Mann-Whitney U test, as appropriate. WBC, white blood cell; RBC, red blood cell; n.s., no significant difference. *All results were within the normal range for pigs [9,24].

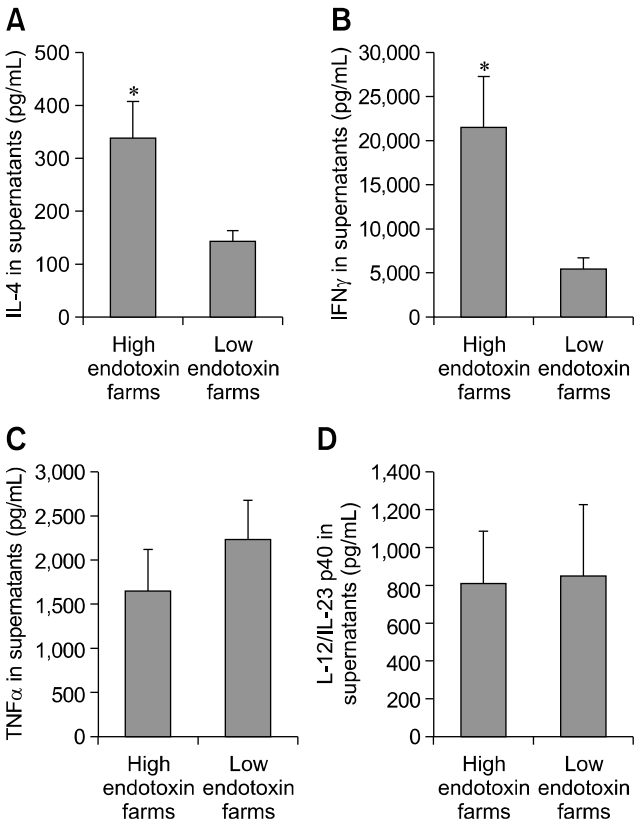

Fig. 1. Interleukin (IL)-4 (A), interferon-gamma (IFNγ) (B), tumor necrosis factor-alpha (TNFα) (C), and IL-12/IL-23 p40 (D) levels in culture supernatants of *in vitro*-stimulated peripheral blood mononuclear cells from pigs sampled at high and low endotoxin exposure farms. Cells were stimulated with concanavalin A/IL-2 for 48 h. The data are expressed as mean ± SE. Significant difference between groups (*$p < 0.05$), as determined by using Student's *t*-test or the Mann-Whitney *U* test, as appropriate.

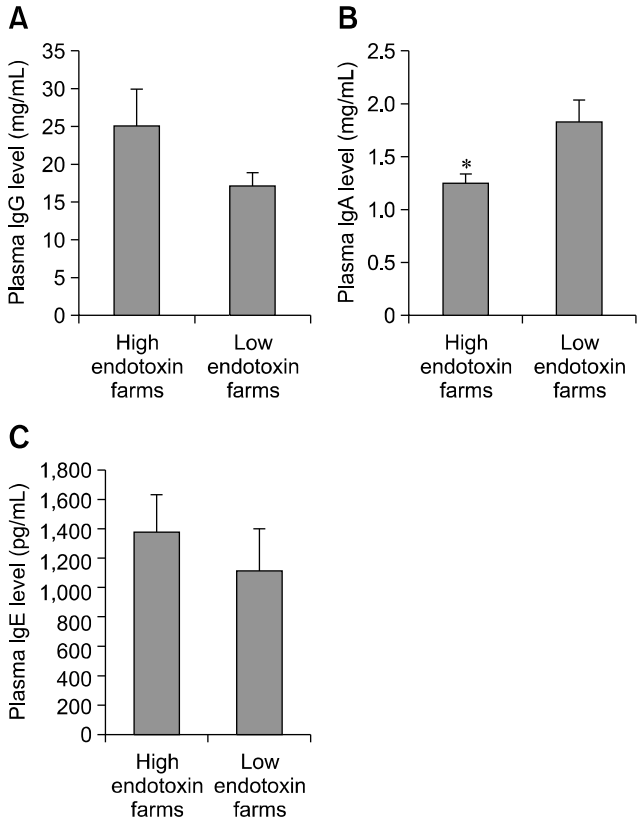

Fig. 2. Concentrations of plasma immunoglobulin (Ig) G (A), IgA (B), and IgE (C) in pigs from high and low endotoxin exposure farms. Significant difference between groups (*$p < 0.05$), as determined by using Student's *t*-test or the Mann-Whitney *U* test, as appropriate.

Discussion

The present study showed that, on average, the ten pig farms that were included in this study had 269.3 EU/m^3 in total and 5.0 EU/m^3 in respirable dust. These levels were particularly pronounced in the high endotoxin exposure farms (defined as $>$ 30 EU/m^3); on average, those farms had 443.18 EU/m^3 in total and 7.22 EU/m^3 in respirable dust. These results are comparable to those in another study of swine barns [3], which reported that swine barns in Lithuania had on average 1,360 ± 500 EU/m^3 in total dust. Schierl *et al*. [36] reported that swine barns in Southern Bavaria had median endotoxin levels of 668.7 EU/m^3 in total and 23.1 EU/m^3 in respirable dust. Several surveys have shown that airborne dusts and endotoxins have detrimental effects on the health of humans working in swine facilities [13,16,21]. However, the adverse effects of endotoxin exposure on animal health and productivity in the natural husbandry environment remain incompletely researched at present. To address this, we assessed the relationships between endotoxin exposure and immunological profiles of intensively reared pigs in Korea.

When endotoxins interact with host cells, it induces them to release proinflammatory cytokines and affects phagocyte differentiation and function [21,29]. Our study showed that pigs living on farms with high endotoxin levels had higher peripheral blood total WBC, neutrophil, and lymphocyte counts than pigs with low exposure to endotoxins. This is consistent with reports showing that animals (including pigs) develop neutrophilia or lymphocytosis when they are experimentally infected with Gram-negative bacteria or are challenged with LPS [4,6,12].

During infection, one of the first cytokines released by macrophages is the proinflammatory cytokine TNFα. Many studies have shown that when pigs are acutely exposed to LPS under experimental settings, their TNFα production is upregulated [18,27,31]. In addition, other studies have shown that repetitive endotoxin exposure can lead to endotoxin tolerance, as indicated by decreased TNFα expression. Wysocka *et al*. [39] showed that when mice are injected with LPS twice in 26 h, or their splenic cells are treated twice with LPS in 20 h, their serum and

Table 3. Correlations between swine husbandry characteristics or endotoxin levels and immunological variables

Immunological variables	Pig age		Endotoxin in total dust		Endotoxin in respirable dust	
	r	*p* value	*r*	*p* value	*r*	*p* value
PBMC* IL-4	0.398	0.006			0.579	0.000
PBMC IFNγ	0.622	0.000			0.697	0.000
PBMC TNFα			0.706	0.001	−0.744	0.000
PBMC IL-1223p40			0.663	0.001	−0.563	0.009
$CD1^+$ B cells					0.555	0.000
Total WBCs			0.496	0.000		
Platelets	−0.679	0.000			−0.361	0.016
Neutrophils			0.420	0.003		
Lymphocytes			0.351	0.016		
Monocytes			0.505	0.000		

PBMCs, peripheral blood mononuclear cells; IL, interleukin; IFNγ, interferon-gamma; TNFα, tumor necrosis factor-alpha; WBC, white blood cell. *The amount of the indicated cytokine produced by concanavalin A/IL-2-stimulated PBMCs isolated from pigs. The correlation coefficients were obtained by calculating Pearson's correlation coefficient. Only statistically significant correlations are shown.

supernatant TNFα levels decrease. Moreover, Castegren *et al*. [5] reported that when pigs are infused for 24 h with endotoxin, and their blood is subsequently treated with endotoxin *in vitro*, their TNFα production decreases. The latter studies are consistent with our observations in which nonspecifically-activated PBMCs from pigs with high endotoxin exposure tended to produce less TNFα than that from the PBMCs of pigs with low endotoxin exposure. Moreover, PBMC production of TNFα correlated negatively with the endotoxin levels in respirable dust. Concerning the positive or negative correlation of TNFα and IL-12/23p40 production from PBMCs with the endotoxin level in total dust and respirable dust, respectively (Table 3), we can offer no clear explanation of this result at the moment. Assuming endotoxin in respirable dust could deeply penetrate into the lungs due to its small diameter (4 μm) compared to total dust (100 μm) [2], endotoxin in respirable dust could have a greater chance of interacting with the immunologic microenvironment in pulmonary alveoli, which may result in a different immune alteration than that from a total dust-mediated immune disturbance. Moreover, in addition to endotoxins, quantitative or qualitative differences in other hazardous components, including microbiological agents or odorous chemicals, may contribute to the different immune modulations mentioned above.

IFNγ and IL-4 typically promote T_H1- and T_H2-mediated immune responses, respectively, and they are mutually antagonistic [14]. Our study showed that the *in vitro*-stimulated PBMCs from pigs with high endotoxin exposure produced significantly more of both of these cytokines than that produced by PBMCs from pigs with low exposure. We also found that PBMC production of IFNγ and IL-4 both associated positively with endotoxin levels in respirable dust. However, high endotoxin exposure was associated with moderate PBMC, skewing toward T_H1-mediated immunity, as indicated by the higher IFNγ:IL-4 ratios in the high-exposure group. This is inconsistent with studies showing that endotoxin exposure promotes T_H2-mediated immunity in broiler chickens and beef cattle [34,35]. This discrepancy may be due to differences in the husbandry environments between our study and those of Roque *et al*. [34,35]. First, the cattle-confinement buildings studied by Roque *et al*. [34] had a lower endotoxin concentration (101 EU/m^3) than that in our swine facilities (443 EU/m^3). Second, while our pigs and the broiler chickens of Roque *et al*. [35] were exposed to similar levels of airborne endotoxin, the broiler chickens were housed under this condition for only 1 month as opposed to approximately 5 months for our fattening pigs. The inconsistent results could be explained by the suggestion that the helper T cell-mediated immunity of intensively reared animals may transit from T_H2 to T_H1 skewness when endotoxin concentrations are particularly high and/or when the animals are exposed to endotoxins for long durations. These possibilities are supported by several studies. First, while humans and rodents exposed to low endotoxin doses exhibit a T_H2 phenotype [1,11,23], exposure to high endotoxin doses appeared to convert T_H2 skewing to T_H1 skewing in skin allergic disease. Second, while T_H2 reactivity predominates in the acute phase of atopic dermatitis, the chronic phase associates with upregulation of T_H1-mediated responses [10,37].

In the present study, the highly endotoxin-exposed pigs tended to have higher plasma IgG and IgE levels than those in pigs with low endotoxin exposure, although no statistical significance was detected. This may reflect a tendency for highly exposed pigs to have higher peripheral $CD1^+$ B-cell counts than that in pigs with lower exposure. This is supported

by the positive correlation between $CD1^+$ B-cell counts and the respirable dust endotoxin levels. Moreover, high endotoxin exposure markedly decreased the plasma IgA levels in pigs. The lower IgA levels may reflect the prolonged exposure to endotoxin: Iqbal *et al*. [15] showed that prolonged endotoxin exposure induces mucosal IgA responses, thereby causing serum IgA molecules to transit from the blood to the mucosal membranes. Notably, Daiwen *et al*. [8] reported that when weanling pigs are experimentally injected with LPS, their serum IgG, IgM, and IgA levels are unchanged. This may indicate that these pigs were only acutely exposed to endotoxin.

Our study showed that when fattening pigs are exposed to prolonged high endotoxin levels (> 30 EU/m^3), it can disrupt their immune homeostasis. Specifically, it skewed the T_H1−T_H2 balance, distorted the peripheral frequency of several immune cells, and dysregulated immunoglobulin production. These observations, especially the altered production of IL-4, IFNγ, and TNFα being correlated significantly with endotoxin levels in respirable dust, suggest that endotoxins may create an immune environment in which animals cannot adequately resist infection by pathogens. Since only 10 pig farms were investigated in our study, generalizations from the results reported herein should be limited. Therefore, systemic investigations should be conducted to elucidate further the molecular mechanisms by which endotoxins in dust can influence the immune profile of pigs. In addition, evaluations of husbandry conditions contributing to indoor endotoxin levels, including humidity/temperature, *ad libitum* feeding system, floor dampness, and slatted floor coverage, are necessary for pig farm managerial purposes.

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Conflict of Interest

The authors declare no conflicts of interest.

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