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Airway responses of healthy farmers and nonfarmers to exposure in a swine confinement building

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Objectives The objective of the study was to determine whether swine farmers continuously exposed to the farming environment react differently to acute exposure than previously unexposed nonfarmers.

Methods Nine healthy nonfarmers, not previously exposed to a farming environment, and eight swine farmers were exposed in a swine confinement building for 3 hours while weighing pigs. Lung function measurements, methacholine challenge tests, and nasal lavages were performed before and after the exposure. Blood samples were drawn repeatedly during the exposure day. Differential cell counts and cytokine levels were analyzed in the nasal lavage fluid and blood.

Results The exposure levels were the same in both groups. Bronchial responsiveness to methacholine increased by a median of 4.0 (25th–75th percentiles 2.2–10.1 among the nonfarmers) and 0.7 (25th–75th percentiles 0.01–3.5 among the farmers) doubled concentration steps. The median serum levels of interleukin-6 increased from 3.8 (25th–75th percentiles <3–5.8) ng/l to 23.7 (25th–75th percentiles 11.6–41.6) ng/l among the nonfarmers and from <3 to 3.8 (25th–75th percentiles 3.1–11.6) ng/l among the swine farmers after the exposure. Swine dust exposure induced a ninefold increase in the total cell counts in the nasal lavage fluid of the nonfarmers, but no significant increase among the swine farmers.

Conclusions The exposure altered lung function and bronchial responsiveness, as well as cell number and cytokines in blood and nasal lavage fluid in previously unexposed nonfarming subjects, whereas only minor alterations were found in the farmers. This finding suggests possible adaptation mechanisms in chronically exposed swine farmers.

Key terms airway inflammation, bronchial responsiveness, cytokines, nasal lavage, swine farmers.

Swine confinement workers have a higher prevalence of respiratory symptoms, such as cough, phlegm, chest tightness, and wheezing, than nonfarming persons (1, 2). In a previous study we showed that nonsmoking, healthy, symptom-free, swine farmers have normal lung function and bronchial responsiveness, but the indices of airway inflammation with increased numbers of neutrophils and higher concentrations of fibronectin, albumin and hyaluronan in bronchoalveolar lavage fluid are higher than for nonfarmers (3). In cross-sectional studies, impaired lung function and increased bronchial responsiveness (4, 5) have been reported for pig farmers when the farmers were compared with referents. Ped-

ersen et al (5) also showed macroscopic signs of bronchial inflammation and an increased number of neutrophils and lymphocytes in the bronchoalveolar lavage fluid of pig farmers.

In healthy nonfarmers, exposure to the environment in a swine confinement facility causes an intense inflammatory response in the upper and lower airways. This reaction is characterized by an increase in inflammatory cells, predominantly neutrophils, and cytokine levels like interleukins 6 and 8 (IL-6 & IL-8, respectively) and tumor necrosis factor alpha (TNF α) in nasal and bronchoalveolar lavage fluid (6–8) and blood (9). The exposure also induces increased bronchial

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responsiveness to methacholine and fever and systemic symptoms such as malaise and chills (10, 11). High exposure to organic dust, especially when contaminated with large amounts of microorganisms, may lead to flu-like disease with fever, chills, malaise, dry cough, mild dyspnea, headache, and muscle pain, usually called organic dust toxic syndrome (12). Vogelzang et al (13) found that pig farmers with less than 5 years of farming experience had a higher prevalence of organic dust toxic syndrome than those who had been working as farmers for a longer time. This difference may indicate possible adaptation mechanisms or a healthy worker effect. Endotoxin is one component that is present in the farming environment, and it is known that repeated exposure to endotoxin results in an attenuation of the inflammatory response, referred to as endotoxin tolerance (14).

We have previously shown that *ex vivo* stimulation of alveolar macrophages from healthy subjects exposed in a swine confinement house *in vivo* yielded a weaker IL-6-, IL-8- and TNF α -response after exposure than it did before the exposure (15). However, two exposures, 8 days apart, in a swine confinement building, induced a similar inflammatory airway response in healthy, previously unexposed subjects even though the dust exposure levels were lower (8).

In our present study, the aim was to compare the airway inflammatory response of swine farmers, who are continuously (daily) exposed to the farming environment to exposure in a swine confinement building, with that of previously unexposed nonfarmers. On the basis of previous studies, we hypothesized that adaptation to this environment may occur when exposure is repeated, and we therefore anticipated a difference in the airway response of the farmers and nonfarmers to exposure.

Subjects and methods

Subjects

Eight swine farmers (8 men), mean age 43 (range 31–54) years, and nine nonfarmers (4 men and 5 women), mean age 24 (range 18–29) years, participated in the study. All the subjects in both groups were healthy non-smokers who denied present or former symptoms of allergy. None of the nonfarmers had previously been exposed to farm dust. The farmers had been working on a swine farm for 15 (range 10–33) years. The preexposure lung function of all the subjects was normal. Vital capacity (VC) was 100 (SD 3)% and 101 (SD 2)% of the predicted values, and forced expiratory volume in one second (FEV₁) was 95 (SD 5)% and 99 (SD 3)% of the predicted values of the referents and swine farmers, respectively. The ethics committee at the Karolinska Institute approved the study.

Study design

All the participants were exposed in a swine confinement building while weighing pigs for 3 hours, one or two from each group being exposed on each occasion. In each pair one subject carried a personal sampler for measuring airborne dust and endotoxins. Approximately 1 week before the exposure, lung function was measured, and a methacholine challenge test was performed. On the exposure day nasal lavage was performed before the exposure and 7 hours after the start of the exposure, when lung function measurements and the methacholine challenge test were also done. Blood samples were drawn in the morning before the exposure and 2, 3, 4, 5, 7, 9, 11, and 24 hours after the start of the exposure. Peak expiratory flow (PEF) was measured in the morning before the exposure and immediately after the exposure.

Exposure measurements

During each exposure one person from each group carried a 25-mm open-phased IOM (Institute of Occupational Medicine) filter cassette and air suction pumps (SKC, Ltd, Dorset, United Kingdom) in the breathing zone at an airflow of 2 l/min. The cassettes were equipped with polycarbonate filters (Nuclepore Corp, Pleasanton, California, United States). Total dust was measured after 24 hours of conditioning by weighing, using a Mettler ME22 balance (Mettler, Greisensee, Switzerland) and reference filters. Endotoxins were extracted from the filters by shaking in pyrogen-free water for 60 minutes, and they were measured after dilution with the chromogen version of the *Limulus amoebocyte* lysate assay [QCL-1000 (Quantitative Chromogenic LAL, BioWhittaker, Walkersville, United States, with *Escherichia coli* 0111:B4 as the standard)].

Symptoms

Symptoms of headache, chills, mental fatigue, muscle pain, and malaise were asked about in a questionnaire and graded on a scale from 1 to 5 (1 = no symptoms, 5 = severe symptoms). The ratings of 4 or 5 were classified as significant. Oral temperature was measured before and 4 and 7 hours after the start of the exposure.

Lung function and airway responsiveness

VC and FEV₁ were measured with a low-resistance rolling-seal spirometer (OHIO model 840, Airco, Madison, Wisconsin, United States) according to guidelines of the American Thoracic Society (16). The reference values of Hedenström et al (17, 18) were used. PEF was measured

with a mini-Wright® peak flowmeter (Clement Clark, Ltd, London, United Kingdom), and the best of three blows was chosen.

The bronchial responsiveness was assessed by a methacholine challenge, performed with inhalation of diluent followed by inhalation of increased doses of methacholine starting at 0.5 mg/ml with a fourfold increase of the methacholine concentration at each step (ie, 0.5, 2.0, 8, and 32 mg/ml). The challenge continued until a 20% decrease in FEV₁, compared with the value obtained after the inhalation of the diluent, had been reached or when the highest methacholine concentration had been inhaled (18). The results were expressed as the cumulative dose causing a 20% decrease in FEV₁ (PD₂₀ FEV₁). If a 20% decrease in FEV₁ was not reached at the highest dose (12.75 mg), a value (15 mg) was assigned for statistical calculation.

Blood leukocyte analysis

Blood samples were collected in EDTA (ethylenediaminetetraacetic acid) tubes. Aliquots were subsequently incubated with the leukocyte cell surface markers CD45-CD14 for 10 minutes (Cytostat/Coulter clone Mo2-RD1/Kc56-FITC, Coulter Electronic Inc, Hialeah, Florida, United States). Thereafter the blood samples were hemolyzed and fixed using a Coulter® Multi-Q-Prep and analyzed by a Coulter 3-part differential white blood cell protocol in a flow cytometer (EPICS PROFILE II, Coulter Electronic Inc, Hialeah, Florida, United States).

Analysis of serum interleukin 6

Blood samples were allowed to coagulate for 1 hour at room temperature before centrifugation. Serum was dispensed in several aliquots, which were kept at -70°C until the analysis. IL-6 was measured with an enzyme-linked immunosorbent assay (ELISA) developed in our laboratory; the procedure has been described in detail by Larsson et al (19), using commercial available antibodies. The anti-human capture monoclonal antibody was MAB206, the detection biotinylated anti-human polyclonal antibody was BAF206, and the recombinant human standard was 206-IL-010 (R&D systems, Europe, Abingdon, United Kingdom).

Nasal lavage

A nasal lavage procedure described by Bascom et al (20) and Pipkorn et al (21) was used with minor modifications. For each lavage, the subject flexed the neck 45 degrees and closed the soft palate, and 5 ml 0.9% sodi-

um chloride was instilled into each nostril with a needleless syringe. After 10 seconds, the subject flexed his neck and expelled the liquid into a plastic basin. The samples were centrifuged, and the supernatant was frozen at -70°C for later analyses. Cells were counted and calculated for the number of cells per milliliter of recovered fluid. Cytochrome-prepared slides were stained with May-Grünwald Giemsa stain for cell differentials. IL-8 in nasal lavage fluid was determined in duplicate using a commercial ELISA test kit (Quantikine, R&D systems, Europe, Abingdon, United Kingdom). The lower detection limit of the assay was 31.3 ng/l. Human serum albumin (HSA) was measured using inhibition ELISA developed at our laboratory (7). The lower detection limit for HSA was 0.11 mg/ml. For duplicated samples an intraassay coefficient of variation (CV) of <10% and an interassay CV of <20% was accepted.

Statistical analysis

The results are presented as the median value (25th-75th percentiles), except for the lung function values, which are presented as means and standard deviations. Student's t-test was used for comparing the lung function data. Within-group comparisons were made using Wilcoxon's signed rank test, and the comparisons between the groups were performed by the Mann-Whitney U test. Correlations were estimated by the Spearman rank correlation test. A P-value of <0.05 was considered significant.

Results

Swine dust exposure

According to the samplers carried by the nonfarmers, the total airborne dust concentration had a median of 20 (25th-75th percentiles 16.4-23.2) mg/m³, and the median endotoxin concentration was 1.4 (25th-75th percentiles 0.9-1.9) µg/m³. The corresponding measures from the farmers were 20 (25th-75th percentiles 18.6-22.3) mg/m³ and 1.0 (25th-75th percentiles 0.8-1.5) µg/m³.

Lung function and airway reactivity

VC did not change in either group after the exposure, while FEV₁ decreased by a mean of 8.1 (SD 10.0)% in the nonfarmers (P<0.05), and 1.3 (SD 5.1)% (P=0.36) in the swine farmers (P<0.05 between the groups) (figure 1). The mean PEF value decreased by 10 (SD 11)%

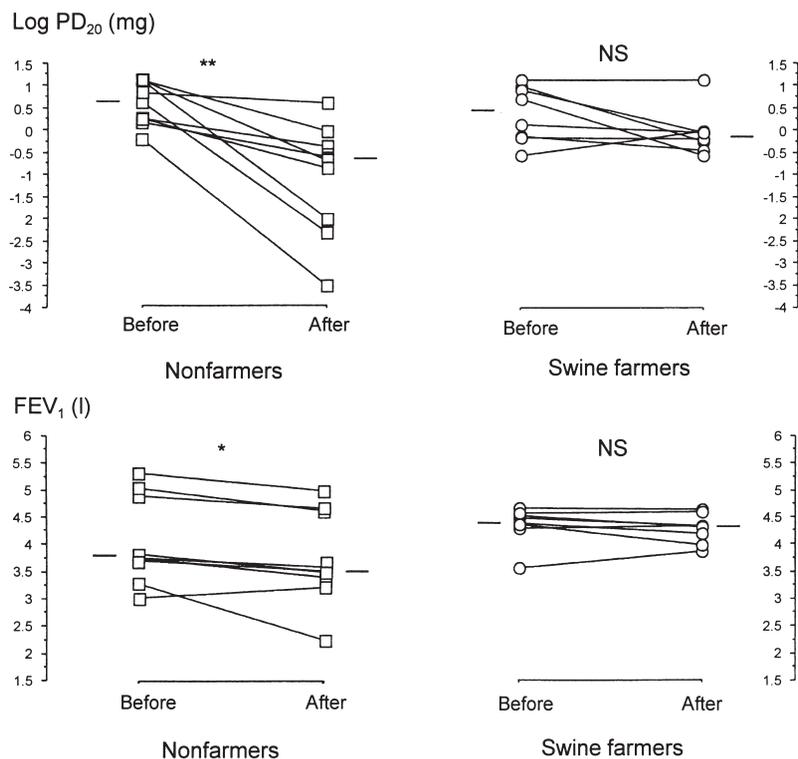


Figure 1. Bronchial responsiveness to methacholine (Log PD₂₀) before and after exposure to swine dust (horizontal lines = median values, **P<0.01 and NS = nonsignificant for comparison before and after the exposure) and forced expiratory volume in one second (FEV₁) before and after exposure to swine dust (horizontal lines = median values, *P<0.05 and NS = nonsignificant for comparison before and after the exposure).

(P<0.05) in the nonfarmers and 4.7 (SD 4.9)% (P<0.05) in the swine farmers, with no significant difference between the groups. Bronchial responsiveness to methacholine increased by a median of 4.0 (25th–75th percentiles 2.2–10.1) doubling concentration steps (P<0.01) among the nonfarmers and by 0.7 (25th–75th percentiles –0.01–3.5) doubling concentration steps (nonsignificant) among the farmers (P<0.05 between the groups) (figure 1).

Symptoms

Exposure induced a median increase of 0.6 (25th–75th percentiles 0.4–0.8)°C in the oral temperature of the swine farmers (P<0.05) and a median increase of 0.3 (25th–75th percentiles 0.2–0.8)°C in the nonfarmers (nonsignificant). Two of the swine farmers and five of the nonfarmers reported symptoms. One farmer reported mental fatigue, and one complained of a headache. In the nonfarmer group, four subjects reported mental fatigue, two had a headache, one had chills, and one reported muscular pain.

Phenotypic evaluation of the blood samples

The numbers of total blood leukocytes and lymphocytes were significantly higher for the nonfarmers in compar-

ison with those of the farmers prior to the exposure (figure 2). The exposure induced approximately a doubling of the total venous blood leukocytes in both groups 7 hours after the start of the exposure, and the level had not returned to preexposure levels after 24 hours. There was no statistically significant difference between the groups (figure 2). The numbers of granulocytes and monocytes were significantly increased 7 hours after the exposure when compared with the preexposure values, the increase of monocytes being significantly greater for the swine farmers than for the nonfarmers (P<0.05). Twenty-four hours after the exposure, the numbers of granulocytes were still significantly higher than prior to the exposure in both groups (figure 2).

IL-6 increased in serum more than fourfold, from a median of 3.8 (25th–75th percentiles <3–5.8) ng/l to 16.0 (25th–75th percentiles 10.6–36.7) ng/l for the nonfarmers 5 hours after the start of the exposure (figure 3). In the farmers IL-6 increased in the blood from <3 ng/l before the exposure to a maximum level of 3.8 (25th–75th percentiles 3.1–11.6) ng/l 5 hours after the exposure (P<0.01 between the groups, figure 3).

Nasal lavage

The exposure induced an almost ninefold increase in the total cell concentration of the nasal lavage fluid (P<0.01)

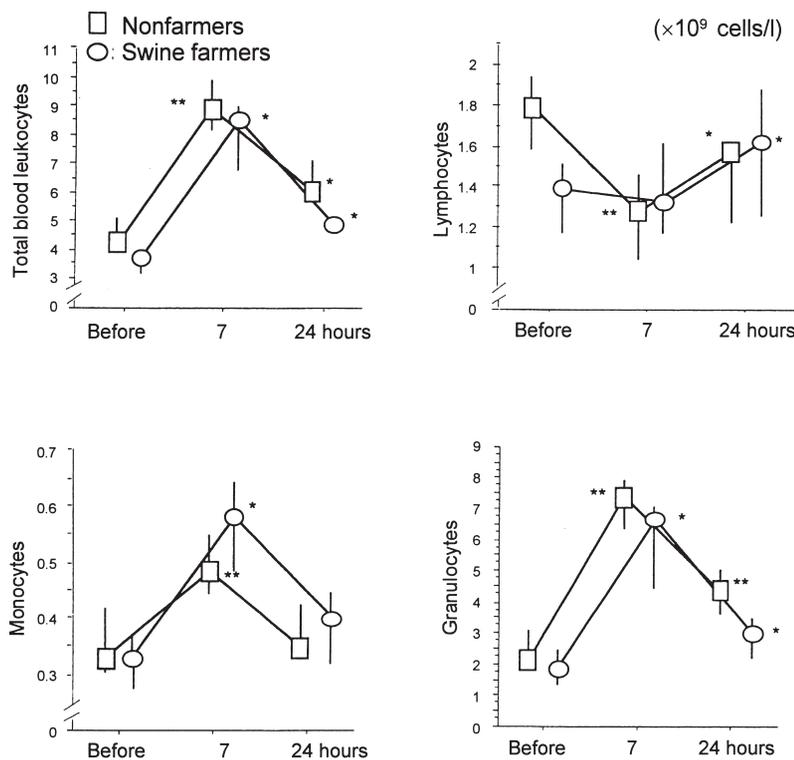


Figure 2. Total blood leukocyte, lymphocyte, monocyte, and granulocyte concentrations in the peripheral blood of the swine farmers and nonfarmers before and 7 or 24 hours after exposure to swine dust. The results are presented as the median and interquartile range (* $P < 0.05$ and ** $P < 0.01$ for comparison before and 7 or 24 hours after the exposure).

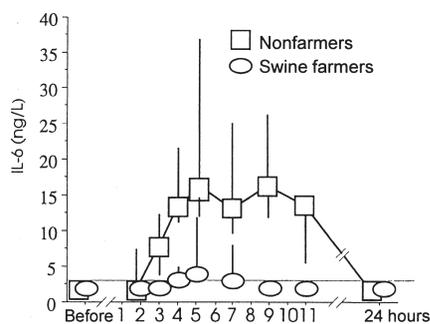


Figure 3. Interleukin 6 (IL-6) concentrations in the serum of the swine farmers and the nonfarmers. The results are presented as the median and interquartile range.

of the nonfarmers, but no significant increase occurred in the swine farmers ($P < 0.05$ between the groups, figure 4). The number of neutrophils in the nasal lavage fluid increased significantly in both groups ($P < 0.05$). The concentration of IL-8 increased significantly after the exposure with no significant difference between the groups ($P = 0.39$, figure 4). There was a significant correlation between the neutrophil and IL-8 increases in the nasal lavage fluid of the nonfarmers ($\rho = 0.79$, $P < 0.05$), but none for the swine farmers ($\rho = 0.57$, $P = 0.16$). The albumin concentration increased signifi-

cantly in both groups after the exposure, with no significant difference between the groups (figure 4).

Discussion

These results confirmed our previous findings that exposure in a swine confinement facility causes an intense inflammatory reaction in the respiratory tract of healthy, previously unexposed nonfarmers (3, 6, 10). In addition, the nonfarmers and farmers responded differently to the exposure. Thus nonfarmers' changes in their lung function and bronchial responsiveness to methacholine following exposure were greater than those of the farmers. Furthermore, the preexposure numbers of total blood leukocytes and lymphocytes were lower in the farmers whose monocyte numbers increased more after the weighing of pigs. The concentration of IL-6 in serum increased in the nonfarmers only, and the increase in the number of cells in the nasal lavage after exposure was greater in the nonfarmers than in the farmers.

Kline et al (22) have shown an interindividual variation with regard to the response of healthy subjects to inhaled lipopolysaccharide (LPS). They found ~15% of the subjects to be sensitive (FEV_1 fall by $\geq 20\%$ after the inhalation of $< 6.6 \mu\text{g}$ of LPS) and ~20% to be hyporesponsive ($< 10\%$ decline in FEV_1 after the

inhalation of a total of 41.5 µg of LPS). They also found that ex vivo stimulation of alveolar macrophages and blood monocytes with LPS from sensitive subjects released higher amounts of both IL-6 and IL-8 than cells from nonresponders did. Thus there are interindividual differences with regard to the response to LPS, and possibly also other agents in the farming environment. The finding of a more-pronounced reaction in the nonfarmers than in the farmers in our study could be the result of a healthy worker effect in that tolerant subjects continue to work in swine confinement buildings. When individual values were analyzed for bronchial reactivity among the nonfarming subjects, three of nine subjects showed a more-pronounced decrease in the PD₂₀ values after exposure, this finding indicating a possible interindividual difference in airway response. However, the number of participants in our study was too low to allow any conclusions to be drawn in this respect.

The weaker inflammatory response of the farmers compared with that of the nonfarmers could also have been due to an adaptation to the environment among the swine farmers. Vogelzang et al (13) found that pig farmers who had worked less than 5 years had a higher prevalence of organic dust toxic syndrome than those who had been working for a longer period, this finding indicating possible adaptation mechanisms. Others have reported attenuated responses (particularly cytokines such as TNFα) after repeated endotoxin exposures, often referred to as endotoxin tolerance or adaptation. A combination of TNFα and IL-1 could mimic the effects of LPS in inducing tolerance in vivo. In guinea pigs the LPS-induced neutrophilic airway response decreased markedly after repeated exposure to LPS (23). However, two exposures, conducted 8 days apart, in a swine confinement building resulted in similar airway inflammatory responses at both times in normal unexposed subjects and thus indicated that one previous exposure is not enough to induce adaptation (8). However, the levels of dust exposure were slightly lower and the endotoxin levels much higher in that study. In cotton mill workers the FEV₁ was decreased on Mondays but not on Wednesdays and Fridays (24).

The inflammatory response of the airways in the nonfarmers seems to be the normal inflammatory reaction to a very strong stimulus, since almost all the previously unexposed nonfarmers exposed in this environment have reacted similarly (6–11). The weaker response in the swine farmers could be a sign of defense mechanisms regulated downwards towards toxic substances in the swine environment. This possibility may be an explanation in the long run for the higher prevalence of respiratory diseases such as chronic bronchitis and obstructive lung diseases found among pig farmers (1, 2, 25). Pedersen et al (5) showed that farmers with inflamed bronchial mucosa (assessed by bron-

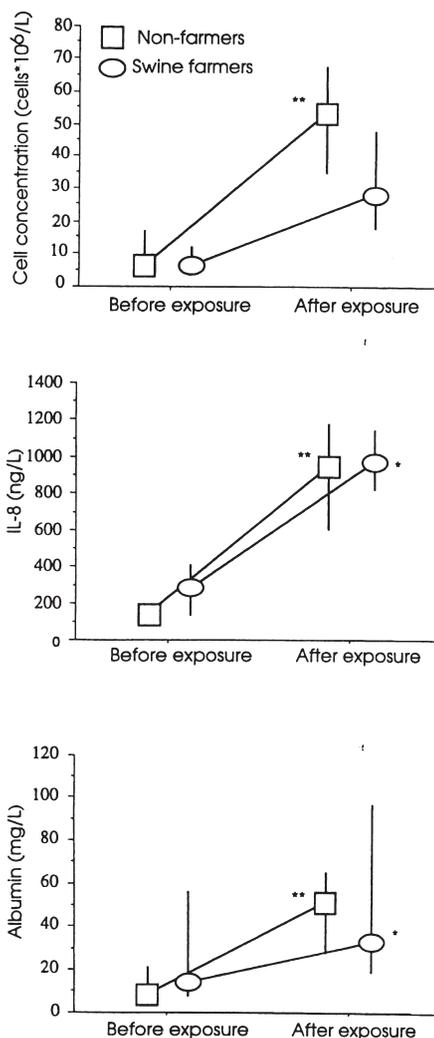


Figure 4. Findings in the nasal lavage fluid before and 7 hours after the start of the exposure to swine dust, measured as concentrations of total cells, interleukin 8 (IL-8), and albumin. The results are presented as the median and interquartile range (* $P < 0.05$ and ** $P < 0.01$ for comparison before and after the exposure).

choscopy) had increased airway responsiveness to histamine and an increased number of neutrophils in bronchoalveolar fluid as compared with farmers with macroscopically normal mucosa. The mechanistic basis for the “switch” from the normal reaction to the inhaled material in the pig farming environment to a chronic disease induced by continuous exposure is unclear. For this knowledge longitudinal studies of healthy farmers or animal studies in a chronic exposure situation are needed.

In our study the gender distribution differed between the two groups, the swine farmers all being men and the nonfarmers being of both genders. Kline et al (22) studied airway responsiveness to inhaled LPS and found

that high sensitivity was more common among women than men. Thus the fact that the swine farmers were exclusively men and the nonfarmers included five women could, in part, explain the difference in airway reaction after the exposure to swine dust.

There was also a difference in the age distribution of the two groups in our study, the swine farmers being older than the nonfarmers. In order to determine the possible influence of age, we analyzed 56 healthy subjects between the ages of 18 and 59 years who had participated in previous studies. We found no relationship between age and the increased bronchial responsiveness to methacholine after exposure in a swine confinement building. The postexposure IL-6 serum levels seemed to increase rather than decrease with age (26) and therefore indicated that the lower increase in serum IL-6 levels after the exposure of the swine farmers (who were older than the nonfarmers) was due to their exposure history rather than to the different age distribution of the groups. In addition, in a study by Bruunsgaard et al (27), it was shown that *ex vivo* stimulation of blood with LPS resulted in significantly lower levels of TNF α and IL-1 β in elderly humans when they were compared with young referents, whereas no difference was detected with regard to IL-6.

Epidemiologic data have shown that multiple myeloma occurs in excess among farmers on pig or poultry farms (28–30). There is evidence suggesting that IL-6 is not only a growth factor, but also an inhibitor of apoptosis in myeloma cells (31). The serum concentration of IL-6 was statistically increased in multiple myeloma patients in a comparison with a reference group (32). Thus chronic exposure to the swine confinement environment, yielding an inflammatory reaction, including increased levels of IL-6 in serum, may contribute to the finding of the increased risk of developing multiple myeloma in farmers.

In conclusion, we have shown that nonfarmers previously not exposed to farming environments have a stronger airway reaction to acute exposure in a swine confinement facility than farmers who are exposed daily to a farming environment. These findings suggest possible adaptation mechanisms in chronically exposed swine farmers.

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References

1. Donham KJ, Haglund P, Peterson Y, Rylander R, Belin L. Environmental and health studies of farm workers in Swedish swine confinement buildings. *Br J Ind Med* 1989;46:31–7.
2. Donham KJ. Health effects from work in swine confinement buildings. *Am J Ind Med* 1990;17:17–25.
3. Larsson K, Eklund A, Malmberg P, Belin L. Alterations in bronchoalveolar lavage fluid but not in lung function and bronchial responsiveness in swine confinement workers. *Chest* 1992;101:767–74.
4. Dosman JA, Graham BL, Hall D, Pahwa P, McDuffie HH, Lucewicz M, et al. Respiratory symptoms and alterations in pulmonary function tests in swine producers in Saskatchewan: results of a survey of farmers. *J Occup Med* 1988;30:715–20.
5. Pedersen B, Iversen M, Dahl R. Bronchoalveolar lavage of pig farmers. *Am J Ind Med* 1990;17:118–9.
6. Larsson B-M, Palmberg L, Malmberg PO, Larsson K. Elevated levels of IL-8 in airway lavage after exposure to swine dust. *Thorax* 1997;52:638–42.
7. Wang Z, Larsson K, Palmberg L, Malmberg P, Larsson P, Larsson L. Inhalation of swine dust induces cytokine release in the upper and lower airways. *Eur Respir J* 1997;10:381–7.
8. Cormier Y, Duchaine C, Israël-Assayag E, Bédard G, Laviolette M, Dosman J. Effects of repeated swine building exposures on normal naive subjects. *Eur Respir J* 1997;10:1516–22.
9. Wang Z, Malmberg P, Larsson P, Larsson B-M, Larsson K. Time course of interleukin-6 and tumor necrosis factor- α increase in serum following inhalation of swine dust. *Am J Respir Crit Care Med* 1996;153:147–52.
10. Malmberg P, Larsson K. Acute exposure to swine dust causes bronchial hyperresponsiveness in healthy subjects. *Eur Respir J* 1993;6:400–4.
11. Larsson KA, Eklund AG, Hansson L-O, Isaksson B-M, Malmberg PO. Swine dust causes intense airways inflammation in healthy subjects. *Am J Respir Crit Care Med* 1994;150:973–7.
12. Malmberg P, Rask-Andersen A, Höglund S, Kolmodin-Hedman B. Incidence of organic dust toxic syndrome and allergic alveolitis in Swedish farmers. *Int Arch Allergy Appl Immunol* 1988;87:47–54.
13. Vogelzang FJ, Van Der Gulden JFW, Folgering H, Van Schayck CP. Organic dust toxic syndrome in swine confinement farming. *Am J Ind Med* 1999;35:332–4.
14. Cavaillon J-M. The non-specific nature of endotoxin tolerance. *Trends Microbiol* 1995;3:320–4.
15. Ek A, Palmberg L, Larsson K. Influence of fluticasone and salmeterol on airway effects of inhaled organic dust; an *in vivo* and *ex vivo* study. *Clin Exp Immunol* 2000;121:11–6.
16. Hedenström H, Malmberg P, Agarwal K. Reference values for lung function tests in females. Regression equations with smoking variables. *Clin Respir Physiol* 1985;21:551–7.
17. Hedenström H, Malmberg P, Fridriksson H. Reference values for lung function tests in men. Regression equations with smoking variables. *Ups J Med Sci* 1986;91:299–310.
18. Malmberg P, Larsson K, Thunberg S. Increased lung deposition and biological effect of metacholine use of a drying device for bronchial provocation tests. *Eur Respir J* 1991;4:890–8.
19. Larsson K, Tornling G, Gavhed D, Müller-Suur C, Palmberg L. Inhalation of cold air increases the number of inflammatory cells in the lungs in healthy subjects. *Eur Respir J* 1998;

- 12:825–30.
20. Bascom R, Pipkorn U, Lichtenstein LM, Naclerio RM. The influx of inflammatory cells into nasal washings during the late response to antigen challenge. *Am Rev Respir Dis* 1988;138:406–12.
 21. Pipkorn U, Karlsson G, Enerbäck L. A brush method to harvest cells from the nasal mucosa for microscopic and biochemical analysis. *J Immunol Methods* 1988;112:37–42.
 22. Kline JN, Cowden JD, Hunninghake GW, Shutte BC, Watt JL, Wohlford-Lenane CL, et al. Variable airway responsiveness to inhaled lipopolysaccharide. *Am J Respir Crit Care Med* 1999;160:297–303.
 23. Snella M-C, Rylander R. Lung cell reactions after inhalation of bacterial lipopolysaccharides. *Eur J Respir Dis* 1982;63:550–7.
 24. Rylander R, Haglind P, Butcher BT. Reactions during work shift among cotton mill workers. *Chest* 1983;84:403–7.
 25. Iversen M, Dahl R, Korsgaard J, Hallas T, Jensen EJ. Respiratory symptoms in Danish farmers: an epidemiological study of risk factors. *Thorax* 1988;43:872–7.
 26. Young DG, Skibinski G, Mason JI, James K. The influence of age and gender on serum dehydroepiandrosterone sulphate (DHEA-S), IL-6, IL-6 soluble receptor (IL-6 sR) and transforming growth factor beta 1 (TGFβ1) levels in normal healthy blood donors. *Clin Exp Immunol* 1999;117:476–81.
 27. Bruunsgaard H, Pedersen AN, Schroll M, Skinhoj P, Pedersen BK. Impaired production of proinflammatory cytokines in response to lipopolysaccharide (LPS) stimulation in elderly humans. *Clin Exp Immunol* 1999;118:235–41.
 28. Pukkala E, Notkola V. Cancer incidence among Finnish farmers, 1979–93. *Cancer Causes Control* 1997;8:25–33.
 29. Wiklund K, Dich J. Cancer risk among male farmers in Sweden. *Eur J Cancer Prev* 1995;4:81–90.
 30. Khuder SA, Mutgi AB. Meta-analyses of multiple myeloma and farming. *Am J Ind Med* 1997;32:510–6.
 31. Gado K, Domjan G, Hegyesi H, Falus A. Role of interleukin-6 in the pathogenesis of multiple myeloma. *Cell Biol Int* 2000;24:195–209.
 32. Urbanska-Rys H, Wiersbowska A, Stepień H, Robak T. Relationship between circulating interleukin-10 (IL-10) with interleukin-6 (IL-6) type cytokines (IL-6, interleukin-11 (IL-11), oncostatin M (OSM)) and soluble interleukin-6 (IL-6) receptor (sIL-6R) in patients with multiple myeloma. *Eur Cytokine Netw* 2000;11:443–51.

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