

Effect of exposure to swine dust on levels of IL-8 in airway lavage fluid

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Abstract

Background – Inhalation of swine dust causes airway inflammation with influx of inflammatory cells, predominantly neutrophils, into the lungs. A study was undertaken to determine whether or not exposure to swine dust induces release of interleukin 8 (IL-8) into upper and lower airways and how this possible release is related to cellular influx. A further aim was to study the relationship between the inflammatory response and swine dust exposure.

Methods – Thirty one healthy, non-smoking, previously unexposed subjects were exposed to swine dust during three hours work in a swine house. Bronchoalveolar lavage (BAL) was performed two weeks before and 24 hours after the exposure (n = 16). Nasal lavage and acoustic rhinometry were carried out 1–2 hours before and seven hours after the start of the exposure (n=31). Exposure measurements were performed with personal sampling equipment.

Results – The exposure led to 19-fold and 70-fold increases in the neutrophil concentrations in nasal lavage and BAL fluid, respectively (p<0.001). In BAL fluid macrophages, lymphocytes and eosinophils increased significantly. The IL-8 levels in BAL fluid increased from <31.3 ng/l to 63 (43–109) ng/l (median (25–75th percentile), p<0.001), and in nasal lavage fluid the concentrations increased from 144 (97–227) ng/l to 1064 (864–1437) ng/l (p<0.001). IL-8 levels showed a significant correlation with the increase in neutrophils in the nasal lavage fluid but not in the BAL fluid. Acoustic rhinometry demonstrated significant swelling of the nasal mucosa. The air concentration of inhalable dust was 23.3 (20.0–29.3) mg/m³, endotoxin 1.3 (1.1–1.4) µg/m³, and muramic acid 0.99 (0.78–2.1) µg/m³.

Conclusions – The concentration of IL-8 increases in BAL fluid and nasal lavage fluid following exposure to swine dust and may be one of the chemoattractants contributing to the recruitment of neutrophils to the nasal cavity and the alveolar space. (Thorax 1997;52:638–642)

Keywords: interleukin 8, bronchoalveolar lavage, nasal lavage, organic dust, endotoxin, peptidoglycan.

Workers in swine confinement buildings have a high prevalence of airways symptoms^{1,2} and in a study using bronchoalveolar lavage (BAL)

it was shown that healthy swine confinement workers have an increased number of inflammatory cells and other signs of an ongoing inflammatory lung reaction.³ In healthy, previously non-exposed subjects it has been established that inhalation of swine dust causes bronchial hyperresponsiveness⁴ and an intense airway inflammation as assessed by BAL.⁵ It seems probable that inhalation of swine dust also causes an inflammatory response in the upper airways although this has not been studied in detail. The cellular reaction to inhaled swine dust in healthy subjects is characterised by a recruitment of inflammatory cells (predominantly neutrophilic granulocytes) to the alveolar space,⁵ and an increase in several cytokines, such as IL-1α, IL-1β, TNF-α and IL-6,⁶ and other inflammatory mediators in the BAL fluid. The mechanism for the recruitment of neutrophils is not known. There are many chemoattractants which may contribute to the influx of neutrophils into the airways such as IL-8,⁷ ENA-78,⁸ LTB₄,^{9,10} and C5a.¹¹ Since swine dust is a very potent stimulus for neutrophil attraction to the airways, the aim of the present study was to investigate whether or not IL-8, one of the probable chemoattractants for neutrophils, is released in the upper and lower airways following exposure to swine dust.

Methods

SUBJECTS

Thirty one non-smoking healthy subjects (16 men) of mean age 31 (range 18–50) years who had not previously been exposed to farm dust participated in the study. All subjects denied present or former symptoms of allergy and airway diseases. They had normal lung function (FEV₁103 (96–109)% and VC 101 (95–108)% of predicted values). All subjects gave their informed consent and the study was approved by the ethics committee at the Karolinska Institute.

STUDY DESIGN

The subjects were exposed during three hours work while weighing swines in a swine confinement building with 600–900 pigs. The participants assisted the farmer and guided the pigs through weighing boxes. Two subjects were exposed at each occasion. Personal samplers were used for exposure measurements. Nasal lavage and acoustic rhinometry were performed in the morning, 1–2 hours before and seven hours after the start of the exposure, when they also answered a questionnaire concerning symptoms. This procedure was performed in

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all 31 subjects with the exception of acoustic rhinometry which, due to technical reasons (equipment out of order during a short period), was performed in only 25 subjects. In 16 of the 31 subjects bronchoalveolar lavage was performed two weeks before the exposure and 24 hours after the start of the exposure.

SYMPTOMS

Symptoms of headache, chills, mental fatigue, muscle pain, and malaise were asked for in a questionnaire. The symptoms were graded according to the severity on a scale of 1–5 (1 = no symptoms, 5 = severe symptoms). Only rates of 4 or 5 were classified as significant. Oral temperature was measured before and repeatedly 4–9 hours after the start of the exposure.

NASAL LAVAGE

A nasal lavage procedure described by Bascom and Pipkorn^{12,13} was used with minor modifications. The subject flexed the neck 45° backwards and closed the soft palate while 5 ml 0.9% NaCl was instilled into one nostril using a needleless syringe. After 10 seconds the neck was flexed forwards and the liquid was expelled into a plastic basin which was placed on ice during processing. The procedure was repeated on the other side. The volume of the combined lavage portions were measured and centrifuged for 10 minutes at 200 g at +4°C and the supernatant was frozen at –70°C until analysis. The pellet was resuspended in 0.9% NaCl with 0.1% human serum albumin and the cells were counted in a Bürker chamber and the number of cells in the recovered lavage fluid was calculated. Cytocentrifuge-prepared slides were stained with May-Grünwald Giemsa stain and 300 cells were counted for cell differentials. Less than 100 cells was considered too few cells to make an accurate differential count.

BRONCHOALVEOLAR LAVAGE

Bronchoscopy was performed through the mouth or the nose with a flexible fiberoptic bronchoscope (Olympus Type 4B2) under local anaesthesia with lignocaine after pre-medication with morphine scopolamine. The bronchoscope was wedged in a middle lobe subsegmental bronchus and 250 ml of sterile saline at +37°C was instilled in five aliquots of 50 ml. After each instillation the fluid was gently aspirated and collected in a siliconised plastic bottle kept on ice.

After filtering through a single layer of gauze, the BAL fluid was centrifuged at 400 g for five minutes at +4°C. Control studies have shown that filtering through gauze does not lead to a loss of cells. The supernatant was kept frozen at –70°C until analysis. The pellet was resuspended in Tris-Hank's balanced salt solution at pH 7.4 and the cells were counted in a Bürker chamber. Smears for differential cell counts were prepared by cytocentrifugation. After staining with May-Grünwald-Giemsa, 400 cells were counted.

ANALYSIS OF INTERLEUKIN 8 IN LAVAGE FLUIDS

The IL-8 concentration in the lavage fluid was measured in duplicate by enzyme linked immunosorbent assay using a commercial ELISA kit (Quantikine, R&D Systems Europe, Abingdon, UK). The lower detection limit of the assay was 31.3 ng/l. For duplicated samples an intra-assay coefficient of variation (CV) of <10% and an inter-assay CV of <20% was accepted.

ACOUSTIC RHINOMETRY

The degree of swelling of the nasal mucosa was estimated by the use of acoustic rhinometry. The method describes the geometry of the nasal cavity by analysing the pattern of reflections of an acoustic signal.^{14,15}

EXPOSURE MEASUREMENTS

The subjects carried 25 mm open phased filter cassettes (IOM) in the breathing zone. The cassettes were equipped with polycarbonate filter (Nuclepore Corp, Pleasanton, California, USA) with a pore size of 0.4 µm and the sampling was performed at an airflow of 1.9–2.0 l/min. The air flow was measured immediately before and after sampling and an average value of the air flow was used for calculation of sampled air volume. Inhalable dust was measured after 24 hours of conditioning by weighing using a Mettler ME22 balance (Mettler, Greisensee, Switzerland) and reference filters.

The filters used for endotoxin analysis were extracted in 10 ml of endotoxin-free water for 60 minutes while rotating. The extracts were centrifuged for 10 minutes at 1000 g and the supernatant was frozen at –70°C for later analysis with the chromogen version of *Limulus ameobocyte* lysate assay (QCL-1000, Endotoxin, BioWhittaker, Walkersville, USA with *E coli* 0111:B4 as standard).

Muramic acid, an amino sugar present only in eubacteria,¹⁶ was measured by gas chromatography-mass spectrometry.¹⁷ This amino sugar is a constituent of peptidoglycan, a cell wall component of both Gram negative and Gram positive bacteria. Aliquots of the filter extracts were transferred to test tubes with Teflon-lined screw caps, dried, and heated overnight at 100°C in 4 M hydrochloric acid. One ml of hexane was added to each tube and after shaking the aqueous (acidic) phase was evaporated, subjected to trimethylsilyl (TMS) derivatisation, and analysed for muramic acid. Muramic acid forms 10–20% of the total peptidoglycan mass. The peptidoglycan content was thus estimated by multiplication of the muramic acid concentration by 100/15.

DATA ANALYSIS

The results are presented as median values with 25th to 75th percentiles. Comparisons were performed by means of Wilcoxon's signed rank test and Spearman's rank correlation was used to estimate correlation. Linear regression was used for illustrating the relation between exposure, cellular response, and IL-8 concentration in the nasal lavage fluid. A p value of <0.05 was considered significant.

Table 1 Numbers of cells and concentration of IL-8 in nasal lavage fluid before and seven hours after the start of exposure to swine dust in 31 healthy subjects

	Before	After	p value
Total cells ($\times 10^6/l$)	5.4 (2.9–15.4)	68.0 (39.2–141)	<0.001
Neutrophils ($\times 10^6/l$)	3.4 (0.33–11)	66.0 (31.4–171)	<0.001
Epithelial cells* ($\times 10^6/l$)	2.9 (1.6–6.6)	0.69 (0–2.3)	NS
IL-8 (ng/l)	144 (97–227)	1064 (864–1437)	<0.001

* Cell differential counts were performed in nasal lavage fluid from 20 subjects due to low cell concentration before exposure. Values are given as median (25th to 75th percentiles).

Table 2 Numbers of cells and concentration of IL-8 in bronchoalveolar lavage fluid before and 24 hours after exposure to swine dust in 16 healthy subjects

	Before	After	p value
Total cells ($\times 10^6/l$)	88 (78–104)	341 (202–494)	<0.001
Neutrophils ($\times 10^6/l$)	1.6 (0.7–2.5)	114 (64–226)	<0.001
Lymphocytes ($\times 10^6/l$)	6.2 (2.4–9.0)	8.8 (5.8–14.5)	<0.05
Alveolar macrophages ($\times 10^6/l$)	84 (68–95)	188 (129–315)	<0.001
Eosinophils ($\times 10^6/l$)	0.09 (0–0.38)	1.6 (0.33–4.8)	<0.01
IL-8 (ng/l)	<31.3	63 (43–109)	<0.001

Values are given as median (25th to 75th percentiles).

Results

FINDINGS IN LAVAGE FLUIDS

The cell counts in nasal lavage fluid are summarised in table 1. The recovery of the nasal lavage fluid was 77 (72–81)% before and 71 (63–78)% after the exposure. The cell concentration increased more than 12 times following exposure to swine dust ($p < 0.001$) and the concentration of neutrophils increased 19-fold ($p < 0.001$). No significant changes in the numbers of lymphocytes, monocytes, epithelial cells, eosinophilic granulocytes, and basophilic granulocytes in nasal lavage fluid were observed. Cell differential counts in nasal lavage fluid could not be performed in 11 subjects before exposure because the cell concentration was too low (table 1).

IL-8 concentrations in nasal lavage fluid increased from 144 (97–227) ng/l to 1064 (864–1437) ng/l ($p < 0.001$, table 1). The cellular increase in nasal lavage fluid, mainly neutrophils, correlated significantly with the increase in IL-8 concentration in nasal lavage ($\rho = 0.54$;

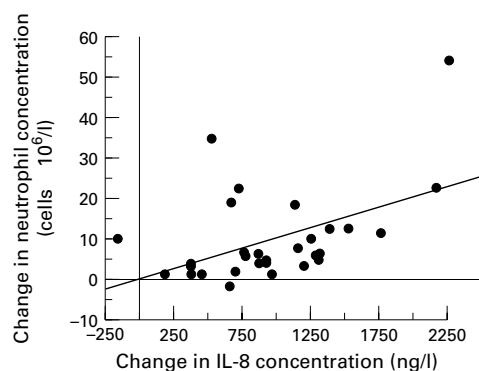


Figure 1 Changes in the concentration of neutrophilic granulocytes expressed as a function of changes in the IL-8 concentration in nasal lavage fluid following exposure to swine dust.

$p < 0.02$, fig 1). No significant correlation was found between the cellular response in nasal lavage fluid and the swelling of the nasal mucosa (as assessed by acoustic rhinometry).

The recovery of the BAL fluid was 69 (65–76)% before the exposure and 66 (60–76)% after the exposure. The cell concentration was almost quadrupled ($p < 0.001$) and the number of neutrophils increased more than 70 times ($p < 0.001$). The numbers of macrophages, lymphocytes, and eosinophils also increased significantly after exposure. The cell counts in BAL fluid are shown in table 2.

In BAL fluid exposure to swine dust induced an increase in IL-8 levels from values below the detection limit (31.3 ng/l) before exposure to 63 (43–109) ng/l after exposure (table 2). No significant correlation between the cellular response (increase in neutrophils, lymphocytes or macrophages) and IL-8 concentration in the BAL fluid was found.

There was no correlation between nasal lavage fluid and BAL fluid with regard to neutrophil influx or IL-8 levels.

EXPOSURE MEASUREMENTS

The concentration of inhalable dust was 23.3 (20.0–29.3) mg/m^3 and the endotoxin concentration was 1.3 (1.1–1.4) $\mu\text{g}/\text{m}^3$. The concentration of muramic acid was 0.99 (0.78–2.1) $\mu\text{g}/\text{m}^3$, corresponding to a peptidoglycan concentration of 6.6 (5.2–14) $\mu\text{g}/\text{m}^3$. No correlation between inhalable dust or airborne endotoxin concentration and cell counts or IL-8 concentration was observed in nasal lavage or BAL fluid. In addition, the IL-8 concentrations in both upper and lower airways did not correlate with peptidoglycan concentration. However, there were significant correlations between peptidoglycan concentration and the increase of neutrophils in BAL fluid ($\rho = 0.66$; $p < 0.02$).

SYMPTOMS AND NASAL FUNCTION

The oral temperature increased by 0.6 (0.3–1.0) $^{\circ}\text{C}$ 5–11 hours after the start of the exposure. Eleven of the 31 participants reported some kind of symptom, of which 10% were chills, 6% headache, 26% mental fatigue, and 6% muscle pain.

Acoustic rhinometry showed a significant reduction in the volume of the first 7 cm in the nasal cavity on both sides. On average the volume decreased by 24% (8.4 (7.3–10.2) ml to 6.4 (5.6–7.7) ml, $p < 0.01$) on the right side and by 15% (9.5 (7.0–11.2) ml to 7.6 (6.3–9.9) ml, $p < 0.05$) on the left side. The minimal cross sectional area also decreased on both sides ($p < 0.05$).

Discussion

These results confirm our previous finding that exposure to swine dust causes an intense alveolar inflammation in healthy, previously unexposed, subjects.^{4,5} In addition, we found that exposure to swine dust causes a significant increase in neutrophilic granulocytes in nasal

lavage fluid and the concentration of IL-8 increased significantly in lavage fluid obtained by nasal and bronchoalveolar lavage. IL-8 is an important chemoattractant for neutrophils^{7,18} and studies have shown this cytokine to be produced by a number of cell types including macrophages,¹⁹ epithelial cells,²⁰ fibroblasts,²¹ endothelial cells,²² mast cells,²³ and neutrophils.²⁴ We have no clear evidence that mast cells are involved in the reaction to swine dust and macrophages are not present in the nasal mucosa. Thus, epithelial cells could be an important source of IL-8 detected in the nasal lavage fluid. The correlation between the increase in IL-8 and increase in neutrophils in the nasal lavage fluid indicates the possibility that inhaled dust induces IL-8 production, presumably from the epithelial cells, and that this cytokine may play an important part in the influx of neutrophils into the upper airways. However, neutrophils are also major producers of IL-8²⁴ and there is a possibility that the relationship between the increase in neutrophils and IL-8 is not causal but rather reflects the influx of neutrophils into the upper airways.

An increase in the concentration of IL-8 was also observed in BAL fluid, but no correlation was seen between IL-8 concentration and the increase in neutrophils. This lack of relationship, and the discrepancy with the findings in the upper airways, may be explained by the fact that the bronchoalveolar lavage was performed later (24 hours after exposure) than the nasal lavage (seven hours after exposure). Stimulation of neutrophils with lipopolysaccharide induces a maximal production of IL-8 after approximately two hours while monocytes maintain a constant secretion rate for 12 hours after stimulation.²⁵ It is not known which cells in the lower airways are responsible for IL-8 production or the time course for IL-8 production following exposure to swine dust. IL-8 production in the lower airways may have peaked at an earlier time point and the response, as assessed by lavage 24 hours after exposure, may not have been properly detected. We have previously shown that swine dust induces production of IL-8 in epithelial cells and alveolar macrophages *in vitro*.^{26,27} Thus, in the lower airways alveolar macrophages and epithelial cells are most likely to be the dominant cell types with regard to IL-8 production.

Chemoattractants other than IL-8 may also be of importance in the recruitment of neutrophils to the airways. Among possible candidates are arachidonic acid metabolites such as LTB₄, predominantly from neutrophils,^{9,10} and 15-HETE²⁸ which is produced by many different cell types such as neutrophils, macrophages, and epithelial cells. We have preliminary data which suggest that exposure to swine dust induces increased levels of LTB₄ in nasal lavage fluid (unpublished observation), but the importance of this finding is difficult to evaluate. The complement factor C5a, which is generated upon activation of the complement system, is also a potent chemoattractant for neutrophils.¹¹ Epithelial neutrophil activating protein 78 (ENA-78) is, like IL-8, a member of the C-X-C chemokine family and is chemo-

tactic for neutrophils.⁸ This protein is produced by epithelial cells, fibroblasts, and monocytes.²⁹ Since many of these alternative factors are produced by cell types that are present and actually dominate the cell population in the lower airways, the relative biological importance of IL-8 as a chemoattractant for recruitment of neutrophils is as yet difficult to evaluate.

The factor(s) in swine dust which cause the airway inflammation is unknown. Endotoxin may be of importance and inhalation of lipopolysaccharide (LPS) has been shown to increase the numbers of neutrophils and lymphocytes in BAL fluid in healthy subjects.³⁰ In the present study the exposure to endotoxin was quite high but no correlation was found between the endotoxin levels and cellular response in airway lavage fluid. The inhalable dust concentration measured during the weighing procedure of the pigs was more than twice as high as during a normal work shift in a swine confinement building.³¹ In a similar study by our group of workers in a poultry confinement building for egg production the dust concentration was less than one quarter of the levels in the present study. We have previously shown that swine dust is a more potent stimulus for IL-8 production in epithelial cells than is LPS.²⁶ Thus, it seems plausible that factors other than endotoxin contribute to the inflammatory reaction to inhaled swine dust.

Feed components (grain dust) may also be of importance in the pro-inflammatory activity of swine dust. Grain dust does not induce IL-8 release in epithelial cells *in vitro*,²⁶ but inhalation of an aqueous grain dust extract causes an acute inflammatory response with an increased concentration of neutrophils and raised levels of cytokines (IL-6, IL-1 β , IL-8, and TNF- α) in BAL fluid.³² We have previously found that inhalation of swine dust induces increased levels of IL-1 β and TNF- α in lavage fluid from upper and lower airways.⁶ IL-1 β and TNF- α induce IL-8 production in many cell types. In nasal epithelial cells mRNA expression and secretion of IL-8 is induced by stimulation by IL-1 and TNF- α .³³ Bronchial epithelial cells are a major source for IL-8 and the production of IL-8 from the bronchial epithelium is substantially amplified by IL-1 β and TNF- α .³⁴ Our own *in vitro* studies of epithelial cells have shown that swine dust is a very potent stimulus for IL-8 release²⁶ while it does not induce release of IL-1 or TNF- α .³⁵ It thus seems probable that the IL-8 response to inhaled swine dust is in part mediated by IL-1 and TNF- α , although it cannot be the sole mechanism. Although not conclusive, these findings indicate that agents in the dust may not only interact directly with the epithelium to increase the production of IL-8, but also indirectly through other cytokines.

Microorganisms, predominantly bacteria, are also important constituents of swine dust and the dominating type of bacteria are of the Gram positive genera.^{31,36} In animal experiments it has been clearly shown that neutrophil recruitment to the airways induced by bacteria (*Pseudomonas aeruginosa*) is mediated

by IL-8.³⁷ The potency of bacteria to induce IL-8 production in epithelial cells has also been shown by others^{38,39} and we have found that Gram positive bacteria are potent stimuli for IL-8 release in a human carcinoma cell line (A549).⁴⁰ In this study we found a significant correlation between the concentration of airborne bacterial peptidoglycan and the increase in neutrophils in BAL fluid. This correlation indicates that the bacterial content of the airborne dust plays a central role in the development of airway inflammation following exposure in a swine confinement building. The lack of correlation between cellular findings and airborne endotoxin also strengthens the suggestion that the Gram positive species are particularly involved in the inflammatory reaction.

In conclusion, this study has confirmed previous findings that exposure to swine dust leads to an inflammatory reaction characterised by a marked influx of neutrophils to the airways. We also found a significant reaction to inhaled dust in the upper airways and increased levels of IL-8 in lavage fluid from both upper and lower airways. The positive correlation between peptidoglycan and the change in concentration of neutrophils in BAL fluid suggests that the presence of microorganisms in the dust may be important in causing the inflammatory response.

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