
Low tumor necrosis factor α levels and neutrophil counts in nasal lavage after mold exposure

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Background: The results of allergy tests against molds usually remain negative in patients with upper respiratory tract and conjunctival symptoms after microbial exposure in a water-damaged building. Most mold-exposed persons report nasal irritation. Immune mechanisms of the nasal symptoms have not been fully elucidated.

Objective: To investigate local inflammatory responses after mold exposure in the upper respiratory tract and the feasibility of nasal lavage in diagnosing work-related exposure.

Methods: Altogether, 26 mold-exposed and 20 nonexposed workers from the same hospital were selected for the present study. The work premises of the exposed workers had detectable moisture and microbial problems. All exposed workers and their nonexposed controls underwent clinical examination, laboratory tests to detect allergy to molds, and nasal lavage. Inflammatory cells and proinflammatory cytokines were measured in the nasal lavage fluid. Nasal lavages were performed again 6 months after a thorough renovation of the building.

Results: In the nasal lavage, the neutrophil count and the level of tumor necrosis factor α in the exposed employees were lower, whereas the macrophage and epithelial cell counts were higher than in the control group. After the renovation, no difference was found in inflammatory response between the study group and the control group. The mean concentration of serum IgG to *Stachybotrys chartarum* was higher in the exposed workers.

Conclusions: These results suggest that exposure to toxin-producing microbial growth in a water-damaged building caused immunosuppression in nasal mucosa, leading to a decrease in neutrophil counts and tumor necrosis factor α levels. Nasal lavage is a suitable method for examining inflammatory responses in work-related mold exposure.

Ann Allergy Asthma Immunol. 2009;102:210–215.

INTRODUCTION

Persons exposed to microbes in water-damaged buildings often report respiratory symptoms.^{1,2} On the basis of the results of the meta-analyses, building dampness and related microbes are associated with approximately 30% to 50% increases in a variety of respiratory and asthma-related health outcomes.³ The risk of adult-onset asthma is related to the presence of visible mold and/or mold odor in the workplace, with an adjusted odds ratio of 1.54 (95% confidence interval, 1.01–2.32).⁴

Secondary infections (eg, sinusitis, bronchitis, otitis media) are more common in individuals exposed to moisture-damaged buildings than in unaffected buildings. General symptoms include fatigue, nausea, arthralgia, and fever.^{5–7} The cause of symptoms and diseases in moisture and mold problems is not yet known with certainty.

Although the prevalence of symptoms is high among exposed individuals, according to our field experience clinical findings among them are much less common. Even though symptoms related to exposure in moisture and mold-damaged buildings resemble allergic symptoms, exposed individuals only seldom have IgE antibodies to microbial antigens in serum, and skin test results are rarely positive to fungal antigens.⁸

Microbe specific IgG antibodies have been used to evaluate exposure to molds. Their significance in evaluating occupational exposure is low^{9,10} because they cannot determine the exact location or even verify the occurrence of exposure.¹¹ Without specific markers, proving that the symptoms of employees are caused by working conditions is problematic even for experienced occupational health personnel.

Because the nasal mucosa is the first target organ to be exposed in water-damaged buildings, and the first symptoms usually appear as nasal irritation, we wanted to examine the nasal mucous membrane in symptomatic workers occupying the water-damaged building. Furthermore, we aimed to determine whether the inflammatory changes were reversible. The feasibility of nasal lavage in a clinical investigation of mold-exposed workers was evaluated. We used a case-control design before and after the renovation.

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Disclosures: Authors have nothing to disclose.

Received for publication August 25, 2008; Received in revised form November 11, 2008; Accepted for publication November 18, 2008.

METHODS

Microbial Exposure

Moisture and mold problems had been present for many years before a thorough renovation in a pediatric outpatient clinic in Southern Finland. The present workplace was a typical pediatric outpatient clinic with rooms for medical consultation, laboratory and skin testing, and pulmonary function testing. Microbes were detected in several locations in the present department. Thus, practically all workers were exposed to water-damage microbes in this area. Small repairs had been performed earlier in the clinic, but they had not offered a lasting solution. Moisture damage had reappeared soon after the construction of the building had been completed.

Air, surface, and material samples for microbiological investigation were collected. The air samples contained microbes, indicating moisture or mold damage: *Streptomyces* (5–10 CFU/m³), *Rhodotorula* (5–10 CFU/m³), and *Aspergillus* (10 CFU/m³) genus microbes. The surface samples contained elevated levels of bacterial growth and moderate levels of fungi (*Acremonium* and *Aspergillus* genera). The material samples contained elevated microbe levels, and the dominant species were the following common moisture damage indicator species: *Aspergillus versicolor* (45–16,292 CFU/g), *Acremonium* (450–26,126 CFU/g), and *Streptomyces* (45–8,776 CFU/g). Also, *Stachybotrys chartarum* (768,182 CFU/g) was found. One of the 3 samples taken for toxin measurements contained mycotoxins: satratoxin (satratoxin G, 0.29 µg/g; and satratoxin H, 0.11 µg/g) and sterigmatocystin (1 ng/g). The renovation was extensive and covered the whole department during which all water-damaged materials were removed. Thus, indoor air sampling was not necessary after the renovation.

The employees at the clinic turned to the hospital's own occupational health services because of an indoor air problem and work-related symptoms. They reported irritation of eyes; irritated, stuffy, and runny nose; hoarse, dry throat; fatigue; and cough. The employees also had recurrent respiratory infections (sinusitis and bronchitis).

Study Population

Overall, 28 employees of the pediatric outpatient clinic participated in the present study. The control group consisted of 20 voluntary employees from other departments within the same hospital with no known moisture damage. Before the present study, 6 exposed employees had been diagnosed as having asthma and 3 with allergic rhinitis, the cause of which still remains unresolved. Exclusion criteria included active infectious disease in the respiratory tract and present mold or moisture problems at home. The possible domestic moisture damage was ruled out in an interview during the first medical examination. The mean ages of the study participants were 48 years (SD, 8.03 years) in the study group and 44 years in the control group (SD, 8.26 years). In the study group, 2 of 28 individuals were men, whereas in the control group the ratio was 1:20. Altogether, 4 of the 28 individuals in the study group and 3 of the 20 controls were daily smokers. The study

was approved by the institutional review board of the hospital, and study participants were enrolled after oral informed consent was obtained.

Clinical Examination and Laboratory Tests

Medical examination was performed in all participants, focusing especially on the nasal mucous membranes and nasal fluid, conjunctiva, pharynx, and skin. Lung auscultation was also performed. The participants were asked whether they had experienced nasal, ocular, or dermal symptoms, cough, or hoarseness attributed to work.

A blood sample was collected from each participant to measure blood eosinophils, total serum IgE, and microbe specific IgE and IgG antibodies to *Acremonium kiliense*, *Aspergillus versicolor*, *Fusarium moniliforme*, *Phoma betae*, *S. chartarum*, and *Thermoactinomyces vulgaris*. Antibody testing was performed with commercially available microbial antigens. Microbial antibodies were defined at the laboratory of the Skin and Allergy Hospital of the Helsinki University Central Hospital (HUCH) with the enzyme-linked immunosorbent assay method (Immuno-CAP; Phadia, Helsinki, Finland).

Pulmonary Function Tests

The pulmonary function tests included flow capacity spirometry, which included forced vital capacity (FVC), forced expiratory volume in 1 second (FEV₁), and the ratio of FEV₁ to FVC. A bronchodilation test was performed with a 0.4-mg (2 × 0.2-mg) salbutamol dose aerosol using a spacer. The histamine provocation test evaluated bronchial responsiveness. The dosage of histamine was 0.02, 0.1, 0.4, and 1.6 mg, following the FEV₁ values and symptoms.

Allergy Tests

Skin prick testing was performed using 12 allergens (birch, alder, timothy, meadow fescue, mugwort, *Cladosporium herbarum*, cat, dog, horse, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and latex). Skin prick testing was performed on the inner forearm, and the skin reaction was read after 15 minutes. The test result was considered positive if the allergen caused a skin reaction of at least 3 mm in diameter and the control solution caused the expected reaction. The test solutions used were commercial extracts (Nordic, ALK-Abello, Round Rock, Texas; 10 HEP [histamine equivalent prick]).

Nasal Lavage

An ear, nose, and throat specialist (A.A.A.) performed the nasal lavage after confirming that the study participants had not used either antihistamine or corticosteroid medication during the previous week. A careful anterior rhinoscopy with a nasal speculum was performed on all patients. The condition of the nasal mucosa and the presence of polyps or other conditions were evaluated. Nasal lavage was performed with a latex urine catheter (Foley 14). The balloon of the catheter was filled with air and pushed gently against the nostril. The nasal passages were flushed with 10 mL of sodium chloride

solution (37°C). After 1 minute of washing, the sample was collected in the syringe, which was immediately placed on an ice bed, and subsequently the sample was frozen. This was done to both nostrils separately, and the samples were pooled. On average, the lavage sample was 7 mL.

Nasal lavage was performed for the first time when the moisture and mold problem was encountered in the hospital on 26 members of the study group and all 20 members of the control group. A follow-up was conducted 6 months after a thorough renovation on 16 members of the study group and 8 members of the control group. The follow-up measurement was performed at the same time of year as the original.

Cytological Staining

An aliquot of the nasal lavage fluid sample was taken aside, fixed in ethanol (1:2), and stored at +4°C until cytospun on microscope slides and stained with PAPA stain. The remaining native nasal lavage sample was centrifuged (10 minutes at 250g in +4°C) and the cells were resuspended into 2 mL of the supernatant, whereas the rest of the supernatant was aliquoted and stored at -70°C for cytokine analysis. Of the resuspended cells, 100 µL were cytocentrifuged (Cytospin; Shandon Ltd, Cheshire, England) on a slide, and the cells were stained with May-Grünwald-Giemsa stain. The cell population on the Cytospin slides was differentiated by counting at least 200 cells under light microscopy. The remaining cells were resuspended in Trizol reagent (Invitrogen; GIBCO BRL, New York, New York) and stored at -70°C for later RNA isolation.

Cytokine Measurements

The supernatants were studied for proteins, myeloperoxidase (MPO), eosinophilic cationic protein (ECP), tumor necrosis factor α (TNF- α), and interleukin 6 (IL-6). MPO and ECP were studied by immunoassays at the HUCH laboratory (Pharmacia MPO radioimmunoassay and CAP System ECP fluorescent-enzyme immunoassay; Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). TNF- α and IL-6 were studied at the Finnish Institute of Occupational Health. The concentrations of TNF- α and IL-6 in the nasal lavage fluid were measured with commercial kits (Quantikine high-sensitivity kits; R&D Systems, Minneapolis, Minnesota) according to the manufacturer's instructions. The detection levels for TNF- α and IL-6 were 0.18 and 0.09 pg/mL, respectively.

Statistical Analysis

Statistical differences in the levels of the inflammatory cells, proinflammatory cytokines, and mold-specific antibodies between the groups were tested using SAS statistical software, version 9.1 (SAS Institute Inc, Cary, North Carolina), and the nonpaired *t* test.

RESULTS

Clinical History and Findings

In the clinical examinations of the 28 exposed employees at the pediatric outpatient clinic, all but 1 reported symptoms relating to indoor air on a weekly basis. The 1 employee who

did not report any symptoms was present at the clinic only a few hours a week. The symptoms appeared at the workplace and disappeared outside the building. During the follow-up nasal lavage performed after the renovation, the exposed study participants reported fewer symptoms, especially irritation symptoms of mucosa. The prevalence of indoor air-related symptoms among employees in the control group did not differ from the general population.¹² No significant difference was found in clinical findings (anterior rhinoscopy, inspection of the throat, eyes and skin, and pulmonary auscultation) among the exposed workers and the control group.

In the pulmonary function test in the study group, an abnormal finding was detected for 2 employees in the study group, and the result of the histamine provocation test was abnormal (FEV₁ decrease of at least 15%) in 6 employees. In the control group, an abnormal spirometry finding was made with 1 and the histamine provocation test was positive for 2 who both had asthma.

Evaluation of Atopy

In skin prick testing to 12 allergens, 10 of 26 employees in the study group and 6 of 18 in the control group reacted to at least 1 test solution. The groups were similar concerning the positive skin test result to common inhalant allergens. The total serum IgE level was above the reference values (0–110 kU/L) with 4 study group and 2 control group members. The total blood eosinophil count was slightly elevated ($0.44 \times 10^9/L$) in 1 member of the study group. In the control group, the blood eosinophil count was normal. Medical history concerning past or present eczema, rhinoconjunctivitis, and asthma was similar in study group and the controls.

Nasal Lavage Cells and Cytokines

In the first nasal lavage, the number of neutrophils in the nasal lavage fluid of the employees in the study group was lower than in the control group ($P = .021$). However, the employees in the study group had more ($P = .028$ and $.014$) columnar epithelium cells and macrophages (Fig 1). The differences in the levels of the nasal lavage eosinophils, lymphocytes, and mast cells were not significant between the study group and the controls. In the follow-up study after the renovation, no differences were found in the number of cells

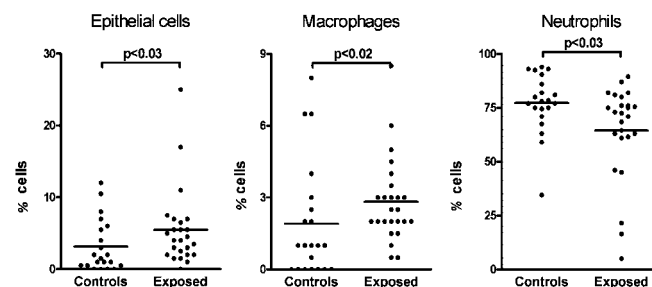


Figure 1. Nasal lavage cell findings in exposed workers and controls before renovation. The cells are represented as values of the total cell amount counted. The line expresses the mean value.

in the nasal lavage fluid between the exposed employees and the control group. No significant differences between the groups were found in the levels ECP and MPO in either study.

The levels of TNF- α in the nasal lavage fluid were significantly reduced in employees working in the moisture-damaged building compared with the control group ($P < .028$). The levels of IL-6 were also slightly reduced in exposed employees compared with the controls (Fig 2A). The follow-up study after the renovation showed no differences in the nasal lavage cytokine levels between the exposed employees and the control group (Fig 2B).

Mold-Specific Antibodies

The mean level of *S chartarum* specific IgG antibodies was higher ($P < .028$) in employees working in the water-damaged building compared with the control group working in a healthy building (Fig 3). No significant differences in the levels of IgG antibodies specific to other molds were found between the 2 groups. Two of the 28 exposed employees demonstrated low levels of mold specific IgE antibodies in serums to *Fusarium* and 1 to *Stachybotrys*. None of the

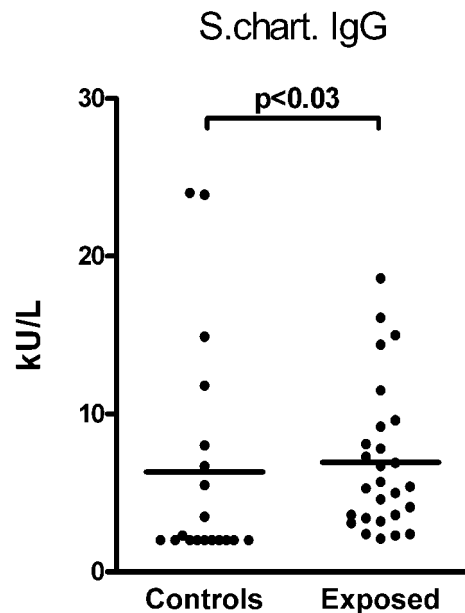


Figure 3. *Stachybotrys chartarum* IgG antibodies in exposed workers and controls before renovation. The line expresses the mean value. S. chart. indicates *Stachybotrys chartarum*.

control group had elevated levels of mold specific IgE antibodies.

DISCUSSION

Working in moisture- and mold-damaged buildings may cause respiratory, eye, skin, and general symptoms, the “sick building syndrome.”^{1-3,12} In the water-damaged building studied, nearly all of the employees at the outpatient clinic were symptomatic based on the indoor air surveys and interviews by the occupational health care. Even though the symptoms of the employees exposed to water-damaged buildings resemble allergic symptoms, the symptomatic individuals are rarely found to have IgE antibodies or test results positive to molds in a skin prick test.⁸ In line with this, only 2 employees tested positive to molds. Furthermore, atopy did not have a significant effect on the prevalence of symptoms. This finding suggests that the symptoms seen in exposed individuals are not likely to have been induced through IgE-mediated mechanisms.

The nasal mucosa is the first barrier for inhaled air; therefore, nasal lavage is possibly a more sensitive indicator for the different irritants in the inhaled air than, for example, examining induced sputum. The cell and cytokine finding from the NAL fluid is a good indicator of the state of inflammation in the nasal mucosa. In an earlier study, staff exposed to molds at a mold-damaged school had significantly higher levels of TNF- α , IL-6, and nitrogen oxide at the end of the spring semester than the nonexposed control group.^{13,14} These inflammation mediator levels decreased to the levels of the control group during the 2.5-month summer holiday. The

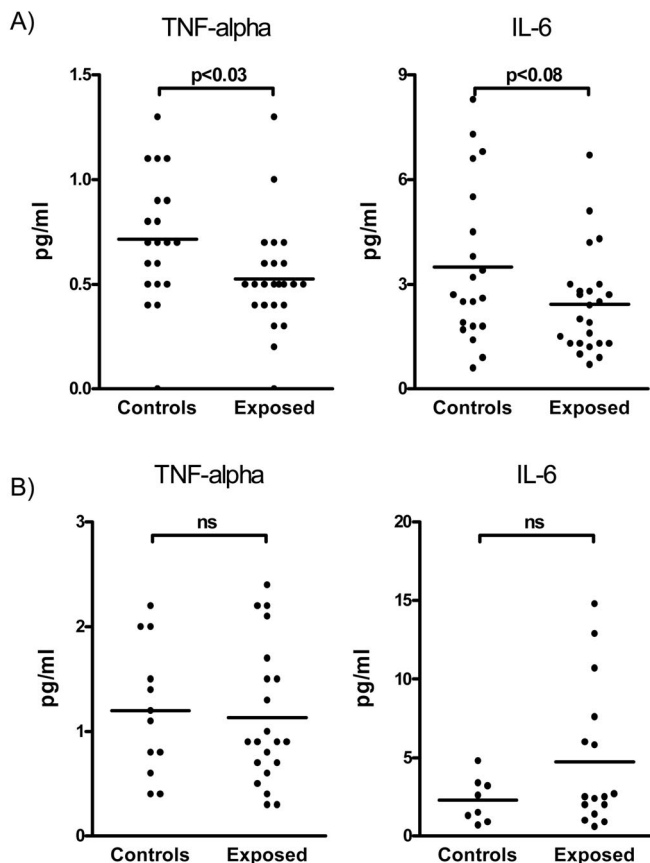


Figure 2. Tumor necrosis factor α (TNF- α) and interleukin 6 (IL-6) in the nasal lavage fluid in exposed workers and controls before and after renovation. The line expresses the mean value.

nitrogen oxide and IL-6 production increased again as the exposure continued during the fall semester. In a Swedish study conducted on workers in a moisture- and mold-damaged building and workers in a control building, the ECP, MPO, and albumin levels in the nasal lavage samples were higher among the workers in the moisture- and mold-damaged office building than among their counterparts in a control building.¹⁵ Another study conducted on saw mill workers found that exposure to microbes does not always lead to inflammatory changes detectable with the nasal lavage method.¹⁶ Thus, it was deduced that the quality of the microbe flora and the microbial products in the environment has an effect on the proinflammatory influence of the microbial exposure.

To examine the inflammatory response in the nasal mucosa in the present study, cellular infiltration and cytokine secretion were investigated from the nasal lavage fluid obtained from the exposed individuals and nonexposed controls. The macrophage and columnar epithelial cell counts were increased, whereas the neutrophil count was reduced in the nasal lavage of exposed employees compared with the control group. In contrast to the nasal lavage study reported previously, TNF- α and IL-6 levels in the nasal lavage fluid of the mold-exposed employees were significantly lower than in the control group. Because the proinflammatory cytokines TNF- α and IL-6 are primarily produced by macrophages, the results indicate that the ability of the nasal macrophages to secrete proinflammatory cytokines is impaired in exposed individuals, even though the number of macrophages in nasal lavage fluid had increased. Interestingly, no significant differences in the number of nasal lavage cells or the levels of proinflammatory cytokines were found between the exposed employee group and the control group in the follow-up study after renovation, suggesting that the impaired immune response was likely attributable to exposure to immunomodulatory substances present in the moisture damage building.

Exposure to specific microbes was evaluated by comparing microbe specific IgG levels between exposed individuals and nonexposed control individuals. Of the 6 microbe strains investigated, only 1, *S chartarum*, could distinguish between the individuals exposed at work and the control group based on IgG antibodies. Large amounts of *S chartarum* were found in the material samples collected from the moisture-damaged building. Another important finding was that 1 of the toxin measurements contained satratoxin, which is a known toxin produced by certain *S chartarum* strains. These results suggest that exposed individuals have been significantly exposed to the toxin-producing strain of *S chartarum*.

In certain situations, exposure to molds leads to an activation of the immune system that can be detected with the nasal lavage method.¹³ However, in some cases exposure to molds does not seem to cause a change in the proinflammatory cytokines of the nasal lavage fluid.¹⁶ In the present study, satratoxin-producing *S chartarum* was found in the building. The exposed individuals showed elevated levels of *Stachybotrys* specific IgG antibodies compared with the control

group. The nasal lavage fluid of the exposed individuals also contained fewer neutrophils than that of the control group. On the basis of these results, exposure to the toxin-producing mold *S chartarum* may lead to the partial suppression of the immune system as indicated by the impaired production of macrophage-derived proinflammatory cytokines and decreased neutrophil counts in the nasal mucosa. However, IgG levels against *S chartarum* were elevated in exposed individuals, suggesting that at least humoral immune response is not impaired. Because the symptoms and inflammatory findings normalized with the termination of exposure, it is anticipated that the 2 are linked. We suggest that exposure to toxin-producing microbial growth, which contains large amounts of *S chartarum*, may result in a partially immunosuppressive condition, which in turn elicits symptoms that are associated with exposure to water-damaged building microbes. Commercial antigens are often weaker in detecting IgG antibodies in patient serum. If crude antigens had been used in the present study, IgG antibodies could have been demonstrated also to other toxin-producing microbes.

In conclusion, the present study shows that nasal lavage is a suitable method for examining mold- and moisture-exposed persons. However, it is important to define both the nasal lavage fluid cells and the proinflammatory cytokines. Depending on the quality of the microbe flora and the microbial products in the environment, the findings in the nasal lavage fluid can suggest immunoactivation or immunosuppression in the nasal mucosa.

ACKNOWLEDGMENTS

We thank Mr Henri Riuttala for his statistical assistance.

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