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Key terms: acoustic rhinometry; aldehyde; dust; eosinophil cationic protein; glucan; indoor air; interleukin 1; interleukin 8; nasal mucosa; rhinostereometry; upper-airway inflammation

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Upper-airway inflammation in relation to dust spiked with aldehydes or glucan

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Objectives Organic dust is associated with adverse effects on human airways. This study was done to investigate whether the addition of β-(1,3)-D glucan or aldehydes to office dust causes enhanced inflammation in human airways.

Methods Thirty-six volunteers were exposed randomly to clean air, office dust, dust spiked with glucan, and dust spiked with aldehydes. The three dust exposures contained between 332 and 379 µg dust/m^3. Spiking with 1 gram of dust was done with 10 milligrams of glucan or 0.1 microliters of aldehydes. Acoustic rhinometry, rhinostereometry, nasal lavage, and lung function tests were applied.

Results After the exposures to dust spiked with the glucan and aldehydes, the nasal volume decreased (–1.33 and –1.39 cm^3 (mean), respectively) when compared with the –0.9 cm^3 after clean air or office dust (P = 0.036 for a difference in decrease between exposures). After 2–3 hours the aldehyde-spiked dust caused a 0.6-mm swelling of the inferior turbinate, and glucan-spiked dust produced a 0.7-mm swelling (P = 0.039 for a difference in the swelling between the four exposures). The preexposure nasal lavage cleaned off the mucosa, and lower cytokine concentrations were found after all of the exposures. For interleukin-8, this decrease in concentration was smaller after the dust exposures spiked with glucan and aldehydes (–2.9 and –25.8 pg/ml, respectively) than after office dust or clean air (–65.9 and –74.1 pg/ml, respectively) (P = 0.042). The nasal eosinophil cell concentration increased after exposure to dust spiked with glucan (P = 0.045).

Conclusions β-(1,3)-D glucan and aldehydes in office dust enhance the inflammatory effects of dust on the upper airways.

Key terms acoustic rhinometry; dust; eosinophil cationic protein; indoor air; interleukin 8; interleukin 1; nasal mucosa: rhinostereometry.

Only a few of the many components of indoor air have been extensively investigated with regard to their health effects. Studies with humans on the effects of mixed exposures are even rarer despite the fact that indoor air is always a complex mixture of substances.

In indoor climate research much attention has been paid to humid buildings and the emission of chemicals from building materials. In moldy buildings β-(1,3)-D glucan has been found in elevated concentrations in the air (1). This is a polyglucose structure originating from plants and microorganisms, including fungi. It has been associated with respiratory symptoms (2), increased peak expiratory flow variability (3), and changes in cytokine release (4–6). In indoor air, the adverse health

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effects of formaldehyde are well known, whereas the effects of other aldehydes are more hypothetical. Aldehydes, as well as other volatile organic compounds (VOCs) in the air, bind to and react with other compounds in the air. We carried out an exposure study comparing the effects of office dust, office dust spiked with β-(1,3)-D glucan, and office dust spiked with aldehydes. In the evaluation of the upper airways, we applied acoustic rhinometry, rhinostereometry, and nasal lavage with assessments of cellular composition, interleukins 1β (IL-1β) and 8 (IL-8), and eosinophil cationic protein (ECP). In the studies of the lower airways, we applied spirometry, peak flow readings, and carbon monoxide (CO) transfer coefficient measurements. All of these methods have previously been used to reveal nonspecific airway reactions to different pollutants. Regarding the interleukins in nasal lavage, the mRNA (messenger ribonucleic acid) expression for these proteins was measured in an attempt to clarify further the extent to which upper airway mucosa was involved in the observed reactions. Our main hypotheses were that, for humans, the inflammatory potential of the dust is augmented by (i) the adsorption of aldehydes on dust and (ii) contamination of the dust with β-(1,3)-D glucan.

Study population and methods

Participant selection

The participants were healthy volunteers found through advertising or in a register of former volunteers. Through a preinvestigation based on inclusion and exclusion criteria, 36 volunteers were recruited. The average age was 30 (range 22–59) years, and the male:female ratio was 14:22.

The study was performed according to the Helsinki Declaration. It was approved by the county Ethics Committee (1999/4486). All of the participants gave their informed written consent.

Preinvestigation

The preinvestigation consisted of (i) a questionnaire, (ii) a medical interview with a clinical examination, (iii) a standard skin prick test with 11 inhalant allergens, (iv) lung function tests, and (v) rhinostereometry with a nasal histamine provocation test. The exclusion criteria were smoking within the previous 6 months, house dust mite or mold allergy, abnormal lung function, loose or false teeth, asthma and nonrespiratory medical or psychiatric conditions precluding safe participation, frequent use of medication, or being non-Caucasian.

Exposures

The volunteers underwent four different exposures in a stainless steel climate chamber. The chamber was cleaned before each exposure session, and all of the participants wore clothing for cleanroom work. During the exposure sessions, the temperature was kept in the range of 22.9–23.0°C, and the relative humidity stayed within the range of 26.8–34.2%. The exposure sessions were always conducted between 0900 and 1300. The first 30 minutes was an acclimatization period with clean air exposure. The exposure was built up for 30 minutes and maintained at equilibrium for 180 minutes. The exposures were clean air, clean air with office dust, clean air with office dust spiked with aldehydes (dust + aldehydes), and clean air with office dust spiked with β-(1,3)-D glucan (dust + glucan). The timing of the clinical investigations before, during, and after exposure is illustrated in figure 1.

Design

Three participants were exposed simultaneously in the climate chamber in sessions separated by 14 days. A total of 12 participants underwent all four exposures during 16 such sessions. Such an "exposure-block" lasted 2 months, and a total of three exposure blocks was necessary for all 36 persons to go through all four exposures. Within the exposure blocks, the exposures were

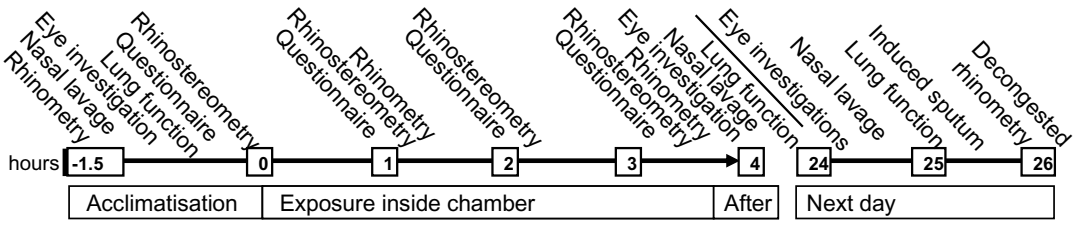

Figure 1. Timing of the clinical investigations in relation to the 3.5-hour climate chamber exposure session. All of the investigations were perfomed once before the exposure session (preexposure between 0 and 1.5 hours before the start) and one to three times during the 3.5-hour exposure sessions or within 30 minutes thereafter (postexposure). Some were repeated the next morning (between 24 and 26 hours from the start of the exposure).

randomly assigned to the participants. The first block started in November 1999 and the last ended in June 2000. This balanced latin-square crossover design leveled out the possible effects of training, day, week, and order of exposure. The investigators and the participants were blinded to the exposures. Those with pollen allergy were tested outside the pollen season.

Clinical investigations

Rhinostereometry. Changes in the swelling of the nasal mucosa were measured directly by use of rhinostereometry (7). This is an optical measuring method performed using a movable microscope on a foundation onto which an individually adapted tooth splint has been also fixed. The surface of the nasal mucosa can be studied through the microscope, which has a small depth of focus. Changes in swelling can be read along the horizontal millimeter scale of the ocular. The accuracy of the method is 0.18 millimeters when repetitive measurements are performed (7). With a lever, the focal plane, in which the mucosa is studied, can be moved sagitally between three parallel positions 1.0 millimeters apart. The readings in the middle of the focal positions were used if available. If unavailable, readings from the first position, with the average difference between the two positions added to them, were used.

During the chamber exposures, rhinostereometry measurements were performed four times. The position of the nasal mucosa was read off repeatedly during each measurement until a stable or no less than five readings had been recorded.

Acoustic rhinometry. The geometry and volume of the nasal cavity was assessed by use of acoustic rhinometry as described by Hilberg et al (8). The measurements were made with the participants seated. The nasal cavities were studied until three reproducible measurements were obtained on both the left and right side. The medians of these measurements were calculated for minimum cross-sectional area. By integration of the area-distance curve, the sums of the volumes 0 to 5 centimeters ($vol_{0\text{-}5}$), and 2 to 5 centimeters ($vol_{2\text{–}5}$) from the nostril were determined.

Nasal lavage. Nasal lavage was done with the participant standing and bending the head forward 30–45 degrees. Through a nose cork attached to a syringe, 5 milliliters of 0.9% sterile saline (body temperature) was introduced into the nostril. After 60 seconds, the fluid was collected, and the lavage repeated in the other nostril. The samples were weighed, had dithiothreitol 30 mM (Acros Organics, Geel, Belgium) added, and were kept for 15 minutes before centrifugation at 3500 revolutions per minute for 10 minutes. The pellet was stained with May-Grunwald Giemsa. A total differential cell count was performed manually under a light microscope. Altogether 200 cells or 20 fields in the microscope were counted, whichever was reached first. For the reverse transcription polymerase chain reaction analysis, 80 microliters of the supernatant was resuspended in 100 microliters of phosphate-buffered saline, kept on ice for 15 minutes, and vortexed with 350 microliters of RLT buffer (Qiagen, VWR International A/S, DK). All of the samples were frozen at –80°C until the analysis.

Immunomarker analyses. IL-1β and IL-8 were measured with a chemiluminiscence enzyme-linked immuno-sorbent assay (Quantiglo, R&D, Abingdon, UK) with detection limits of <0.4 pg/ml (IL-1β) and 0.8 pg/ml (IL-8). ECP was measured with a Pharmacia CAP System1 (ECP FEIA) by Pharmacia & Upjohn, Copenhagen, DK. The detection limit was 0.5 µg/l. All of the means were calculated from duplicate analyses.

Lung function. Spirometry was performed according to guidelines of the American Thoracic Society (9) using a Vitalograph Compact II, pressure differential type spirometer (Vitalograph, Buckingham, UK).

According to published recommendations (10), the participants were instructed in how to record peak expiratory flow with mini Wright peak flow meters (Clement Clarke International Ltd, London, UK). Peak flow was recorded at waking and at 1200, 1800 and 2200 for 1 week before and after each of the exposures.

Measurements of the transfer factor of the lung for CO ($T_{L,CO}$) and the CO transfer coefficient (K_{CO}) were carried out by means of the single breath-holding technique with a Morgan Transfertest Model C (PK Morgan, Chatman, UK) as described by Cotes et al (11).

Dust generation

Dust was collected outside the pollen season by standardized vacuuming (12) in office buildings without known indoor climate problems. The dust was homogenized, mixed, and divided into three subsamples. One subsample was not treated further; another was spiked with a solution of aldehydes in methanol (1:10:100 volume ratio of *n*-hexanal, *n*-nonanal, and *n*-decanal) at 0.1 ml/g dust, and the third was mixed with β-(1,3)-D glucan (Curdlan, Wako Chemicals, GmbH) at 10 mg/g dust. The amount of aldehydes added to dust was calculated from adsorption isotherms extrapolated from available data for formaldehyde and acetaldehyde. The dust + aldehydes and dust + glucan mixtures, in the following referred to as spiked dusts, were stirred for 12 hours. An acoustic generator was used to generate exposures from the dusts into the chamber. The mean

concentration of dust measured by personal samplers (Gilian Hi Flow pump, Sensidyne Inc, FL, USA) during the exposures was less than 20 μg/m^3 for clean air and between 332 and 379 μg/m^3 for the dusty exposures. All three dust exposures had similar dust size distributions with ~70% of the dust in the 1- to 5-micrometer range. During all four types of exposures, the gaseous concentrations of total volatile organic compounds were between 71 and 76 μg/m^3, and the three aldehydes were below 6 μg/m^3. Airborne glucan was analyzed with the use of a limulus coagulation assay (by R Rylander, Göteborg, Sweden). It was measurable only during the dust + glucan exposures at a mean of 16.8 (range 4.3–38.6) ng/m^3.

Statistical analysis

A repeated-measures general linear model was used to evaluate the relationship between the outcome variables and the exposures. Atopy, nasal histamine responsiveness, and exposure block were controlled by entering them into the model as co-factors. If the exposure block showed no effect and did not interact with other variables, it was omitted from the model. Separate evaluations were performed, either using measurements of the exposure days only or using all available measurements, including those from the days following the exposures. Preexposure differences in the rhinostereometry measurements were leveled out by the subtraction of the preexposure reading from all of the readings. Predicted means, standard errors, and 95% confidence intervals (95% CI) were derived from the statistical model with data from the exposure days only. They were used for calculations of the mean changes in the outcome variables. Spearman's rank correlation was used for the correlation tests, as the original data were, in some cases, not normally distributed. SPSS version 11.0 (SPSS Inc, Chicago, IL, USA) was used for the analyses.

Results

Rhinostereometry

Data from the 32 participants with valid readings from the zero or middle focal position during all four exposure sessions were analyzed. There was an interaction between time and exposure (P = 0.039), and this

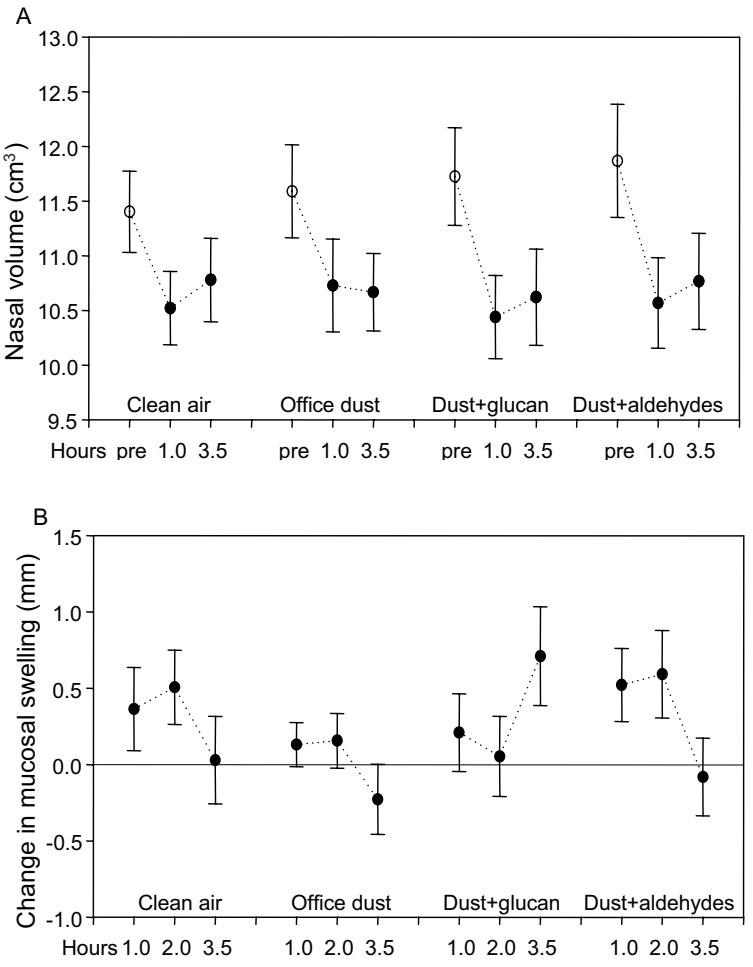

Figure 2. Changes in nasal mucosal swelling in healthy volunteers at different times during four different exposure sessions. Figure A plots the nasal volume 0 to 5 centimeters from the nostril, as measured by acoustic rhinometry shortly before (pre-) the exposures, after 1 hour of exposures (during), and after 3.5 hours at the end of the exposures. Figure B plots the mucosal changes on the right inferior turbinate 1, 2, and 3.5 hours after the start of the exposures (with the preexposure levels set at zero, as represented by the horizontal line). The data are unadjusted residual means from the general linear statistical model. (● = measurements during or immediately after the exposures, ○ = measurements before the exposures).

interaction indicated different time courses of mucosal swelling during the different exposures. As illustrated in figure 2, the nasal mucosa showed the greatest swelling after 3.5 hours of exposure to dust + glucan (mean 0.71 mm, 95% CI 0.05–1.37). During exposure to dust with aldehydes, we observed swelling after 1 hour (0.52 mm, 95% CI 0.03–1.01), and 2 hours (0.59 mm, 95% CI 0.07–1.18), but not after 3.5 hours. During exposure to clean air, we observed swelling after 2 hours (0.51 mm, 95% CI 0.01–1.01). Pairwise comparisons between the recordings revealed only the following two exposure-related differences in rhinostereometry: after 2 hours, dust + aldehydes caused a 0.64-millimeter greater swelling than dust + glucan ($P = 0.013$), and after 3.5 hours dust + glucan caused a 0.88-millimeter greater swelling than dust alone ($P = 0.024$).

Acoustic rhinometry

The nasal volume was higher preexposure than during and after any of the exposures, including clean air ($P < 0.0005$) (figure 2). During the exposures, the vol_{0-5} changed –1.39 cm^3 (95% CI -1.87– -0.92) for dust + aldehydes and -1.33 cm^3 (95% CI -1.76– -0.90) for dust + glucan. During exposure to clean air and office dust, these changes were smaller (mean -0.86 cm^3, 95% CI -1.20– -0.53, and –0.89 cm^3, 95% CI -1.35– -0.43,

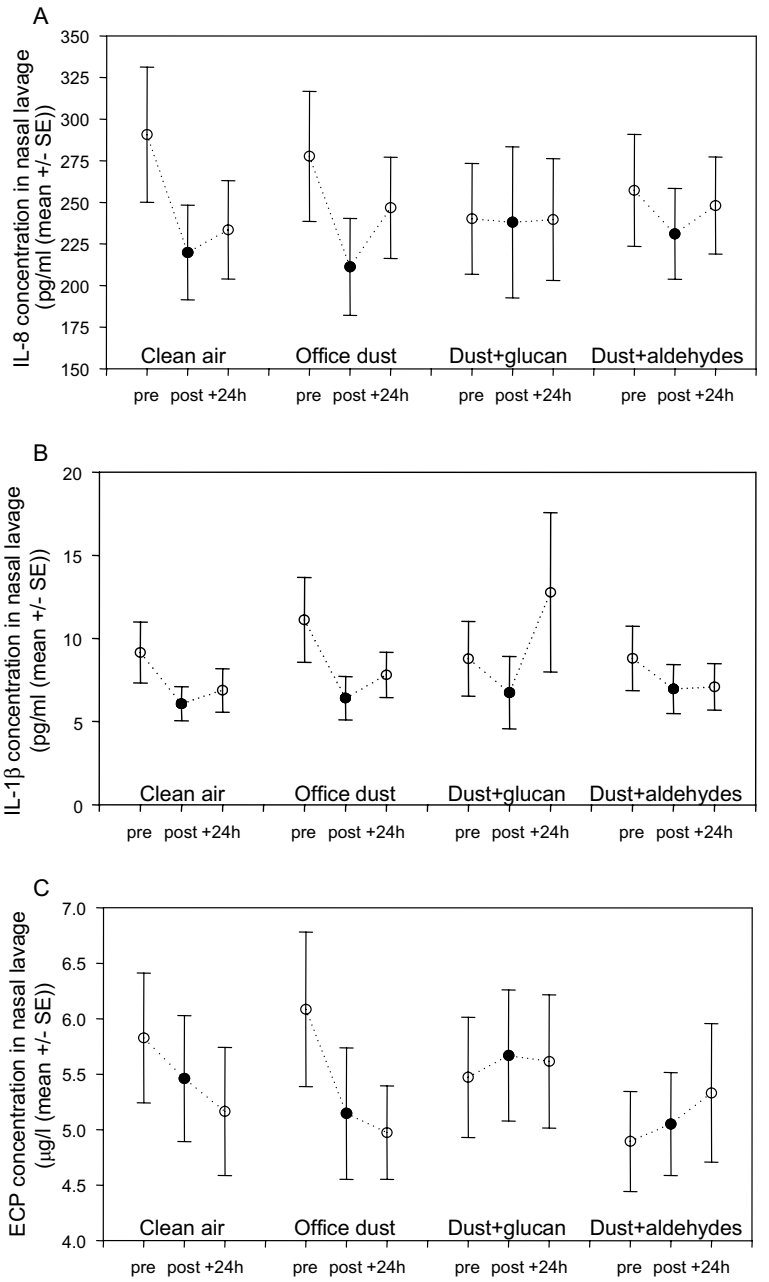

Figure 3. Concentrations, in the nasal lavage, of interleukin-8 (IL-8) (A), interleukin-1β (IL-1β) (B), and eosinophil cationic protein (ECP) (C) shortly before (pre), immediately after (post), and the morning after (+24 hours) the end of four different exposure sessions for the healthy volunteers. The data are unadjusted residual means from the general linear statistical model. (● = measurements immediately after the exposures, ○ = the measurements before and the morning after the exposures).

respectively). There was an interaction between time and exposure (P = 0.036) that indicated different time courses of nasal volume changes during the different exposures. The changes in vol_{2-5} (P = 0.053) and in the cross-sectional area (P = 0.091) tended to show the same pattern of interaction between time and exposure as the changes in vol_{0-5}. Immediately after the exposure, the nasal volumes and cross-sectional area were unchanged compared with what was observed during the exposure.

Nasal lavage fluid

The concentrations of IL-1β, IL-8, and ECP were lower in the nasal lavage fluid sampled immediately after the exposure than in the preexposure samples (figure 3, see p 378). However, after dust + glucan (and for ECP after dust + aldehydes) exposure, these mediators did not tend to decrease. The next morning, the concentration of the mediators in nasal lavage fluid still tended to be lower than the preexposure levels, except after the spiked dust exposures, in which they reached the preexposure levels or, in the case of IL-1β after dust + glucan, they exceeded the preexposure levels.

The greatest change in the IL-8 concentration in nasal lavage fluid from pre- to postexposure was measured after exposure to clean air (mean -74.1 pg/ml, 95% CI -123.3– -25.0), followed by exposure to office dust (mean -65.9 pg/ml, 95% CI -116.3– -15.4). The change was smaller after the dust + aldehyde exposure (mean -25.8 pg/ml, 95% CI -67.3–15.7) and negligible after the dust + glucan exposure (mean -2.9 pg/ml, 95% CI -81.1–75.4). There was an interaction between time and exposure (P = 0.042) that indicated different changes in the IL-8 concentration during the different exposures.

The changes in the IL-1β and ECP concentrations in nasal lavage fluid resembled those of IL-8, the spiked dusts tending to yield smaller pre- to postexposure differences than clean air and dust alone. For IL-1β the changes were small after exposure to clean air (mean -3.4 pg/ml, 95% CI -6.3– -0.7), office dust (mean -4.9 pg/ml, 95%CI -8.4– -1.5), dust + glucan (mean -2.0 pg/ml, 95%CI -4.1–0.1), and dust + aldehydes (mean -2.0 pg/ml, 95% CI -5.0–1.0). For ECP the changes from pre- to postexposure were as follows: -0.39 µg/ml (95% CI -1.47–0.69) for clean air, -0.97 µg/ml (95% CI -2.50–0.55) for office dust, 0.18 µg/ml (95% CI -0.94–1.30) for dust + glucan, and 0.20 µg/ml (95% CI -0.57–0.97) for dust + aldehydes.

In contrast to the decrease in the cellular mediator concentrations from the preexposure levels, the cell counts in nasal lavage fluid did not show a pattern of changes in relation to time. The highest mean count of 223 (SE 134) eosinophils/ml emerged immediately after the dust + glucan exposure. The mean number of eosinophils per milliliter immediately after the other exposures was lower being, 5 (SE 4) after clean air exposure, 65 (SE 27) after office dust exposure, and 84 (SE 55) after the dust + aldehydes exposure (P = 0.045 for a difference between exposures). There was no difference in the eosinophil counts between the exposures the next morning when the concentrations were close to the preexposure levels. We did not observe any exposure-related changes in the concentrations of other cell types, nor did we see any changes in the concentrations of mRNA for IL-1β or IL-8 (not shown).

Lung function

The spirometry and peak flow readings changed very little from the pre- to postexposure measurements. The changes were not related to the exposures. From the pre- to postexposure the changes in the transfer factor and coefficient were not affected by the exposures. When measurements from the next morning were included in the model, exposure effects for the CO transfer coefficient (P = 0.001) and a tendency for the transfer factor (P = 0.056) appeared. This result was due to a decrease in the transfer coefficient the morning after the exposure to spiked dust, as shown in figure 4. Pairwise comparisons showed that the morning after the dust + glucan ex-

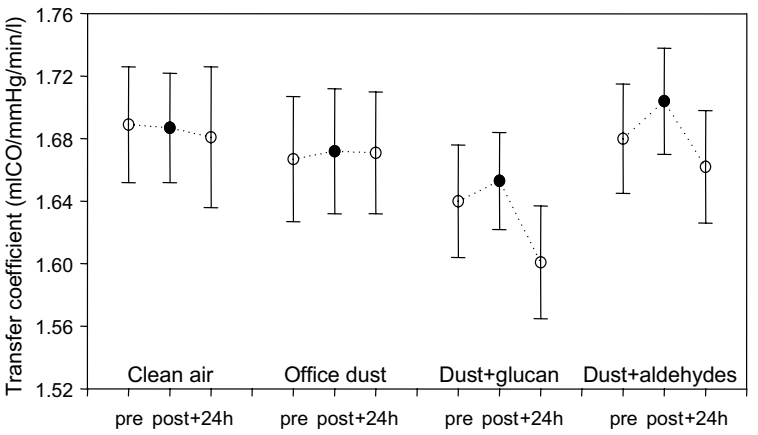

Figure 4. Changes in the pulmonary transfer factor of carbon monoxide (CO) at different times in relation to four different exposure sessions for 36 volunteers. The data are unadjusted residual means from the general linear statistical model. (● = measurements during or immediately after the exposures, (○ = measurements before and the morning after the exposures).

posure, the mean CO transfer coefficient was 3.9% (95% CI 3.1–4.4%) lower than after the exposure to office dust alone.

Discussion

To our knowledge, this is the first experimental exposure study of the inflammatory effects on human airways from dust combined with glucan or aldehydes. Several of the investigations revealed stronger inflammatory nasal effects for the spiked dust than for the other exposures.

An amplified swelling of the nasal mucosa was revealed by acoustic rhinometry. This swelling emerged after the exposure to both of the spiked dusts, and it was supported the by rhinostereometric measurements. Previously, acoustic rhinometry has disclosed nasal mucosal swelling in relation to dampness (13), water damage (14), formaldehyde (15), and high levels of dust (16). In a study, in which aqueous solutions of curdlan (the β-glucan also used in this study) were instilled directly into the nostrils of recycling workers, an increase in nasal volume was observed (17). The effect of pure curdlan may well be nasal mucosal decongestion, as opposed to our observation of an enhanced congestive effect of dust combined with particulate curdlan. This finding parallels the observation for guinea pigs that curdlan has no effect alone, but it amplifies the increase in the number of leukocytes in the lungs after the inhalation of endotoxin or tobacco smoke (18, 19).

We observed no change in the IL-8 concentration after spiked dust exposures as opposed to the decreasing IL-8 concentration after exposure to clean air and dust alone. This relative increase in IL-8 in nasal lavage fluid was the highest after the dust + glucan exposure, with a similar tendency after aldehydes-spiked dust. Tendencies towards relative increases in IL-1β and ECP after spiked dust exposures were also observed. The effect was masked by a washout effect due to the nasal lavage performed in the morning. Such a washout effect was also described in a study on waste composters, in which nasal IL-8 correlated with glucan levels (5). In that study, a mean glucan level of 1.29 μg/m^3 was observed with maximal values over 30 μg/m^3. The concentrations found in buildings (including moldy homes) have been lower, varying between approximately 5 ng/m^3 and 106 ng/m^3 (1, 20). Long term exposures in this range have been found to be associated with congested nose (21), fatigue (20), and dry cough and itching skin (22).

Elevated levels of other biomarkers (ie, myeloperoxidase and lysozyme) have been observed in the nasal lavage fluid of persons exposed to organic dust. The experiment was designed to avoid allergen exposure yielding nonspecific reactions rather than Th2-like responses. We, therefore, chose to measure preinflammatory cytokine IL-1β and chemokine IL-8, both of which are inducible by curdlan in vitro (23). Nasal ECP has been measured in several studies of organic dust, and increases have previously been observed after exposure to volatile organic compounds (24). All three cellular mediators analyzed in nasal lavage fluid tended to increase during or after the exposures to the spiked dust. Obviously, if a number of other cellular mediators had been assessed, we might have been able to understand the inflammatory response in more detail.

Curdlan has also been shown to induce IL-8 mRNA in human whole blood (23). Studies with waste workers have revealed IL-8-associated increases in neutrophils in nasal lavage fluid (5, 25). In one study this response, as well as swelling of the nasal mucosa, was associated with exposure to glucan and fungal spores (25). Our study sustains the hypothesis that IL-8 genes are activated during nasal exposure to curdlan and dust, the result being increased levels of IL-8 and subsequent attraction of neutrophils. However, we did not observe changes in the mRNA content of nasal lavage fluid during this study.

The immunomodulatory potential of different glucans varies much and may depend on their bio-availability (26). Little is known about which glucans have actually been found in most studies, and care must be taken when the observed effects are compared. Furthermore, lack of standardized methods for assessing glucan may hinder comparisons between reported glucan concentrations in different studies. Some authors have suggested that glucan may be a proxy for fungal exposure and that the effects ascribed to it in reality may be caused by other factors (3). In our study we did, however, find effects of curdlan, a β-(1,3)-D glucan.

The irritant effects of lower aldehydes have been subject to detailed study, and both allergic and carcinogenic effects have been explored. An irritant effect of formaldehyde in concentrations at or below recommended limits in indoor climate has previously been observed (27). Little has been published on the effects of higher aldehydes or mixtures of them. Great variations have been reported for the indoor concentrations of volatile organic compounds. In homes, exposures are mixed and do not consist of just a few components, as our experimental exposure did (28). In a Swedish report on patterns of volatile organic compounds in indoor air, a combination of eight such compounds was associated with problem buildings whose dwellers more frequently complained of symptoms (29). Five of these compounds were aldehydes.

Ours is not the first experimental exposure study with β-(1,3)-D glucan. In two studies, one with

volunteers exposed for 4 hours to curdlan at 210 ng/m^3 and the other with the application of aqueous solutions of grifolan treated with sodium hydroxide at 30 ng/m^3, no significant effects on lung function were observed (30, 31). A third study revealed nonsignificant increases in airway ECP and tumor necrosis factor α followed by decreases in systemic inflammatory markers 72 hours after β-(1,3)-D-glucan exposure (32).

In a previous study performed in our climate chamber with pure aldehydes identical to those used in our current study (up to 300 μg/m^3), no significant changes in nasal volume were found (33).

Most of the dust propagated in our study was in the respirable size range and should have reached both the upper and the lower airways. We did not observe much exposure-related change in the lower airways. The most likely explanation for this result is that the effects (if any) were small, and the methods we used were not sensitive enough. Furthermore, the spiked dust exposures were not high in comparison with what has previously been reported. However, a late decrease in the CO transfer coefficient was observed. Such a decrease has earlier been reported after a 4-hour inhalation of endotoxin (34).

The large number of investigations and, thus, of possible associations between exposures and outcomes, increased the risk of spurious findings. A tendency towards lower preexposure levels of IL-8 and ECP before the spiked dust exposures appeared, as can be seen in figure 3. The nasal volumes also tended to be the highest prior to the spiked dust exposures. These unexplained tendencies towards differences in prechallenge levels may have increased the likelihood of changes apparently caused by the spiking being observed. However, all of the emerging significant changes in outcomes, including those for IL-1β and rhinostereometry, for which prechallenge levels were not obviously favorable for our hypothesis, indicated inflammatory effects for the spiked exposures. As we used a general linear model for comparisons between exposures rather than simple paired tests, the possible effect of differences in the prechallenge levels was minimized. Furthermore, higher cytokine concentrations correlated with more nasal mucosal swelling (not shown). We have no reason to believe that our results are random findings. Instead, we believe that our study has revealed small, but real, effects despite the fact that the large number of clinical investigations disturbed each other, as seen with the washout effect of nasal lavage.

In experimental exposure studies, it is only feasible to study exposures within a range of hours. We exposed volunteers for 3.5 hours and may not have been able to identify changes that would have appeared during a typical workday or at the end of a workweek. Nevertheless, the data revealed that the effect of dust was increased when it was combined with other components. A clinical implication of the finding relates to the combination of dust with other contaminants. In this study we used glucans and aldehydes that are ubiquitous in ambient air. With both of these spikings, we demonstrated an increased effect on inflammation. It is not possible to predict what the effects of continuous exposure at lower doses or exposure in combination with numerous other agents would be. Therefore more effort should be given to the total burden of contaminants rather than to the single component itself, as has often been the case. Acoustic rhinometry is a simple and promising tool in this respect if used repeatedly with the same persons (35).

In conclusion, we would like to propose that an enhanced inflammatory effect of dust on the upper airways exists when dust is combined with β-(1,3)-D glucan or with aldehydes in the air. Hence, in studies of indoor air effects, it should be recognized that the properties of dust may change the dose–response relationship.

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