

WORK RELATED ACUTE AND (SUB-)CHRONIC AIRWAYS INFLAMMATION ASSESSED BY NASAL LAVAGE IN COMPOST WORKERS*

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Abstract: In the autumn of 1995, repeated pre- and post-work-shift nasal lavages (NAL) were performed in 14 compost workers (3 weeks on Mondays and Fridays) and 10 controls (one Monday) to assess the relation between bioaerosol exposure and airways inflammation. Total cell counts, leukocytes and inflammatory mediators (MPO, ECP, IL8, NO, albumin, urea and uric acid) were determined in NAL. Mean personal dust and endotoxin exposures ranged from 0.4-3.1 mg/m³ and 50-1000 EU/m³, respectively. A cross-shift increase in total cells and mediator levels (except ECP, albumin and uric acid) was observed in the NAL of workers while in controls there was a cross-shift decrease, presumably due to a wash out effect. Total cells, MPO, IL8, NO and albumin in NAL of workers measured on Mondays after two exposure free days were substantially elevated compared to controls and were more elevated at 'high' than at 'low' endotoxin exposures. These results indicate that occupational exposure in compost workers causes acute and (sub-)chronic non-immune or type III allergic inflammation in the upper airways.

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INTRODUCTION

The introduction of the recycling industry in recent years in developed countries has resulted in new and poorly defined health risks, mainly due to exposures to high levels of microorganisms and their toxins. Many workers involved in 'waste handling' and 'composting' household waste experience respiratory and systemic influenza-like symptoms (shivering, fever, joint pain, malaise) [5, 6]. Some case reports exist on the occurrence of allergic diseases such as extrinsic allergic alveolitis (type III allergy), allergic bronchopulmonary aspergillosis and asthma [2, 6]. In addition, non-allergic airways inflammation may occur as well.

In this study we used nasal lavage to assess airways inflammation in workers involved in household waste composting. Nasal lavage is a relatively new technique, allowing the assessment of upper airways inflammation and elucidation of the pathophysiological mechanisms involved. It is a non-invasive, safe and simple technique that is tolerable for most individuals and has proven its feasibility up to now mainly in experimental exposure studies but also in a limited number of population studies [3, 4]. We assessed acute and (sub-)chronic inflammation in compost workers by performing, during a period of three weeks, pre- and post-shift nasal lavages, on Mondays and Fridays. Workers were compared with controls lavaged prior to and after work only once on

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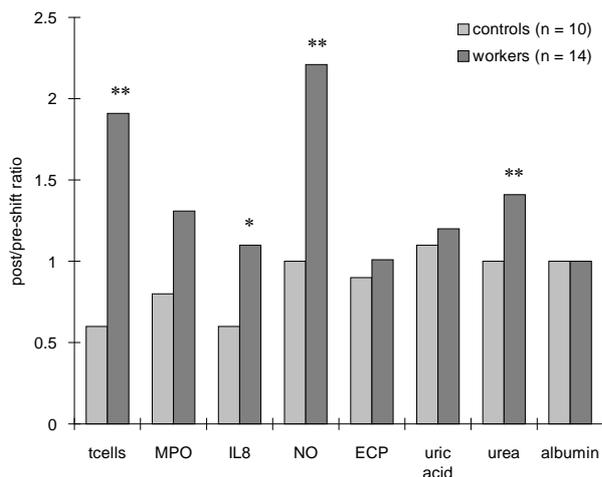


Figure 1. Group median post/pre-shift ratios for various inflammatory markers in nasal lavage (NAL) of compost workers and controls. Abbreviations: tcells, total cells; MPO, myeloperoxidase; IL8, interleukin-8; NO, nitric oxide; ECP, eosinophil cationic protein; * $p < 0.1$, ** $p < 0.05$. All NO levels in both pre- and post-shift NAL of controls were non-detectable (< 100 pmol/ml).

Monday. In the nasal lavage various cell types, and humoral biomarkers of inflammation were determined. Results indicated acute and (sub-)chronic non-immune or type III allergic inflammation in compost workers but not in controls.

MATERIALS AND METHODS

During three weeks on Mondays and Fridays in the autumn of 1995, repeated full shift personal inhalable dust exposure was monitored in 14 composting workers by using PAS-6 samplers at 2 l/min. Endotoxin was assayed in dust extracts with a kinetic chromogenic LAL-assay [1]. Ambient microorganism concentrations were determined using Andersen samplers loaded with Tryptone Soy Agar (TSA), TSA with crystal violet or Dichloran Gelatin 18 (DG18) agar dishes to culture total bacteria, Gram-negative bacteria and molds respectively. Air samples were taken at a flow rate of 28.3 l/min for 1 minute. In case culture plates were overloaded (> 400 cfu per plate) indirect measures were applied using petri dishes loaded with glycerol-gelatin dilution medium that allows dilution prior to culturing on TSA or DG18 plates.

At the same time that personal exposure was monitored, repeated pre- and post-shift nasal lavages were performed in the 14 compost workers (Mondays and Fridays for three weeks). A control group consisting of 10 university staff members and students, received nasal lavage only on one Monday prior to and after work. For the lavage, 5 ml sterile PBS, heated to 37°C , was instilled in each nasal cavity with a 10 ml polystyrene pipet, held for 10 seconds, and then expelled into a centrifuge tube. Lavage fluids were centrifuged for 10 minutes at $250 \times g$ and supernatants were used for the measurement of the inflammatory markers: myeloperoxidase (MPO), eosinophil cationic protein (ECP), interleukin-8 (IL8) and nitric oxide (NO), albumin, urea and uric acid. The latter

3 markers are indicative for exudation due to increased permeability. Part of the pellet was fixed in an equal volume of 4% formalin and the total number of cells per ml was counted. The remainder of the pellet was used for cytopins which were stained with May-Grünwald Giemsa staining for cell differentials (neutrophils, eosinophils and epithelial cells). MPO, ECP and IL8 were measured by RIA, FIA and EIA, respectively. NO was measured using a nitric oxide analyser 280 (NOA 280; Sievers) extended with a purge-vessel. Albumin concentrations were determined on a COBAS FARA and uric acid and urea on a COBAS BIO clinical chemistry analyzer (F. Hoffmann-La Roche, Basel, Switzerland).

In order to avoid dependency in the data, repeated NAL measures per worker were treated as one observation by using the median value of the repeated measurements in the statistical analyses. Thus only 14 observations instead of the available 84 were used. Repeated exposure measurements were also reduced to one exposure measure per worker by using the geometric mean of the 4–6 exposure measurements. Workers and controls were compared by using group median levels based on the 10 observations of the controls and 14 observations of the workers. Differences between workers and controls were tested with a non-parametric Kruskal Wallis test unless stated otherwise.

RESULTS

Geometric mean personal dust exposures in the 14 workers ranged from 0.4 – 3.1 mg/m^3 ($n = 76$) with incidental levels above 10 mg/m^3 , and geometric mean endotoxin exposures ranged from 50 – 1000 Endotoxin Units (EU)/ m^3 ($n = 63$) with incidental exposures of more than 3500 EU/ m^3 . Mean ambient microorganism levels were high in the compost hall with mold and total bacteria counts of more than 1×10^5 cfu/ m^3 (maximum possible count for direct method) and Gram-negative bacteria levels of $10,000$ cfu/ m^3 ($n = 5$). Indirect measures using glycerol-gelatin dilution media indicated total bacteria and mold levels of 1×10^9 and 1×10^6 cfu/ m^3 ($n = 5$) respectively.

All 14 compost workers were males with a mean age of 28.2 (SD 4.2) and 9 out of 14 smoked. Work duration at the compost plant ranged between 2 and 30 months. The 10 controls consisted of 6 males and 4 females ($p < 0.05$; Fisher's Exact test) with a mean age of 29.2 (SD 8.2) and only one of the controls smoked ($p < 0.01$; Fisher's Exact test).

In total 188 nasal lavages were performed of which 168 were performed in workers and 20 in controls. Approximately 6 ml (60%) of the lavage fluid was recovered with no significant difference between workers and controls. Median post/pre-shift ratios for workers showed a cross-shift increase in total cells and most inflammatory markers in the NAL (except ECP, albumin and uric acid), while in controls a cross-shift decrease was observed in almost all NAL parameters, most likely due to a wash-out effect (Fig. 1). Total cells consisted mainly of

neutrophils and epithelial cells but almost no eosinophils. Median levels of total cells, MPO, IL8, NO and albumin in NAL of workers measured on Monday prior to work after two exposure free days were elevated (3500 cells/ml, $p < 0.05$; 53 ng/ml, $p < 0.2$; 155 pg/ml, $p < 0.05$; 217 pmol/ml, $p < 0.05$; 72.2 $\mu\text{g/ml}$, $p < 0.05$; respectively) compared to controls (900 cells/ml, 14 ng/ml, 35 pg/ml, < 100 pmol/ml; all below detection limit, 62 $\mu\text{g/ml}$). ECP was not elevated, urea and uric acid only slightly ($p > 0.2$). Thus, in addition to acute cross-shift inflammatory effects, also (sub-)chronic airways inflammation was demonstrated in the compost workers.

Based on the individual geometric mean endotoxin exposure over 4–6 repeated measurements, workers were divided into a 'high' exposure group ($n = 7$), with geometric mean exposures greater than the median group exposure level of 460 EU/m³, and a 'low' exposure group ($n = 7$) with geometric mean exposures of less than 460 EU/m³. Pre-shift NAL inflammatory markers were in most cases more elevated in the 'high' exposed workers than in the 'low' exposed workers who still had higher levels of inflammatory markers than the controls. Comparison of 'high' and 'low' dust exposed workers showed a similar trend. No clear trend with exposure was observed for cross-shift inflammatory effects.

No significant difference in cross-shift and pre-shift NAL was observed between Mondays and Fridays. Inflammatory response determined in pre-shift NAL seemed somewhat suppressed in smoking workers compared to non smoking workers (NS).

DISCUSSION

Acute and (sub-)chronic airways inflammation was demonstrated in compost workers involved in household waste composting. The (sub-)chronic airways inflammation seemed to be associated with bioaerosol exposure. Microorganisms and their constituents such as endotoxin may be the etiological agents involved. Differences in smoking habits between workers (65% smokers) and controls (10% smokers) could have contributed to the observed differences in airways inflammation. This, however, does not seem likely since smoking was associated with lower concentrations of the inflammatory response markers and thus the actual difference between exposed workers and controls might have been even larger, if the number of smokers had been similar.

Since neutrophils, MPO, IL8 and NO but not eosinophils and ECP seem to play a key role in the inflammatory response, it is suggested that occupational exposure in compost workers may induce a non-immune-specific or a type III allergic inflammation. This finding needs to be confirmed in a larger study.

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