

Wheat Flour Exposure Results in Recruitment of Inflammatory Cells in the Lungs of Healthy Individuals

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Background Flour dust in bakeries is known to cause allergic as well as nonallergic respiratory symptoms. Fungal α -amylase is a commonly used baking additive that has been shown to have allergenic properties. The aim of this study was to investigate any effects on bronchoalveolar lavage (BAL) cells and peripheral blood lymphocytes (PBL) of healthy individuals exposed to airborne wheat flour dust with or without fungal α -amylase added.

Methods Fifteen subjects were exposed during 1 hr in an exposure chamber, ten individuals to wheat flour alone and five with α -amylase added. BAL was performed 2–6 weeks before and 1 day after the exposure. BAL cells were differentially counted and flowcytometric analysis of the expression of activation, adhesion, and subset markers on alveolar macrophages (AM) and T cells in BAL fluid and peripheral blood were carried out.

Results Exposure to wheat flour dust increased the total number of cells in BAL fluid from 75.4 (i.q. range 70.4–104.1) to 127.4 (92.1–187.4) cells $\times 10^6/L$, $P < 0.01$. There was a significant difference in the change of total BAL cell concentration between the study group exposed to wheat flour only ($n = 10$; increase with $91.9 \times 10^6/L$) and the group exposed to wheat flour with the baking additive fungal α -amylase ($n = 5$; decrease with $5.4 \times 10^6/L$). The exposure level of respirable dust was lower in the group that received α -amylase and the increase in BAL cell concentration showed a positive correlation with the concentration of respirable dust in the exposure chamber ($r = 0.80$, $P < 0.001$). The phenotypic analysis of AM indicated an influx of monocytic cells.

Conclusions The results indicate that the concentration of respirable dust, but not α -amylase, is of importance for the recruitment of inflammatory cells to the peripheral airways in healthy individuals exposed to wheat flour dust. Am. J. Ind. Med. 44:75–82, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: airway inflammation; wheat flour; α -amylase; exposure chamber; bronchoalveolar lavage

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INTRODUCTION

Flour dust in bakeries is known to cause allergic as well as non-allergic occupational respiratory symptoms and several studies have shown that bakers run an increased risk of developing such symptoms [Brisman and Järholm, 1995; Karjalainen et al., 1998; McDonald et al., 2000]. Wheat flour dust consists of mainly proteins and starch but also enzymes added to improve its baking characteristics. The most commonly used enzyme in the baking industry is α -amylase of *Aspergillus oryzae*, which can have allergenic properties [Tikkanen et al., 1996; Baur and Posch, 1998; Nieuwenhuijsen et al., 1999]. Although sensitization to components in the flour dust seems to be the main cause of bakers' asthma, many bakers with airway symptoms are not sensitized to either flour allergens or baking additives such as α -amylase. The importance of respiratory irritation rather than allergic reactions has therefore been discussed [Cullinan et al., 1994, 2001; Houba et al., 1996; Baur et al., 1998].

Very few studies have investigated the cellular response in humans exposed to flour dust. Górski et al. [2000] showed an increase in the percentage of eosinophils and basophils in nasal and bronchoalveolar lavage (BAL) of occupationally sensitized bakers following flour dust-inhalation. Also, inhalation to grain dust has been reported to cause both nasal and bronchial inflammatory responses in grain workers as well as in atopic and non-atopic individuals not previously exposed to grain dust [Blaski et al., 1996a,b; Becker et al., 1999].

The objective of this work was to investigate the cellular response, both in the lungs and the blood of healthy individuals following whole-body exposure to flour dust. Ten of the 15 participants were exposed to wheat flour dust alone and five to flour dust with fungal α -amylase added. Bronchoscopy with BAL was performed 2–6 weeks before and 1 day after the exposure. BAL cells were analyzed by differential counts and phenotypical analyses of AM and lymphocytes from BAL fluid and peripheral blood were performed using flowcytometry.

MATERIALS AND METHODS

Subjects

Fifteen healthy nonsmoking volunteers (eight females) with a mean age of 32 (range 22–52) years participated in the study. All subjects underwent a physical examination including chest radiograph and blood routine examination, 2 weeks before the study. None had experienced respiratory tract infection during the last month prior to the investigation or had a history of respiratory tract disease. All individuals were characterized with regard to atopy (history and total IgE) and sensitization to wheat flour was measured by radioallergoabsorbent test (RAST). All subjects were found

to be non-atopic and non-sensitized to wheat flour. The participants gave their informed consent and the local ethics committee approved the study.

Experimental Design

The subjects were exposed to wheat flour dust for 1 hr in a whole-body exposure chamber built and constructed especially for an even and continuous generation of airborne dust [Lidén et al., 1998]. Ten of the subjects were exposed to wheat flour alone while five were exposed to a mixture of wheat flour and the baking additive fungal α -amylase (Fungamyl 2500 BG, Novo nordisk A/S, Bagsvaerd, Denmark). The wheat flour used was of standard quality grade normally used in a large Swedish bakery. To reach the exposure concentrations chosen, a series of preparatory tests were carried out. An air concentration of approximately 5 mg/m³ (total dust) was chosen as a suitable particle concentration corresponding to the Swedish threshold limit value (TLV) for organic dust at the time of this study. This value also corresponds roughly to an exposure level in bakeries measured during an 8-hr workday [Burdorf et al., 1994; Tikkanen et al., 1996]. For the exposure session with wheat flour together with α -amylase, the airborne level of α -amylase in the chamber was targeted to be approximately 40 ng/m³ at a total dust concentration of 5 mg/m³. Small portions of α -amylase were mixed with wheat flour of the same batch as was used in the exposures with wheat flour alone. Mixing and homogenization was standardized and performed in a Turbula T2c (WAB AG Maschinenfabrik, Basel, Germany). During the progress of the study a new TLV value for inhalable flour dust (3 mg/m³) was introduced in Sweden and, therefore, we measured inhalable dust levels for five of the individuals. All exposures were performed at air exchange rates in the chamber of 15–17 per hour.

Sampling and Analysis of Wheat Flour Dust and α -Amylase in the Chamber

Dust samples were collected in two positions close to the breathing zone on either side of the subject during each exposure. Total dust was sampled in 37 mm open-faced cassettes (Millipore holder, Millipore, Bedford, MA) and inhalable dust (defined as particles with cut-point 100 μ m), was measured in IOM (IOM: Institute of Occupational Medicine, Edinburgh, Scotland) samplers (SKC Ltd., Dorset, UK) at flow rate 2 L per minute (lpm). Respirable dust, defined as airborne particles with cut-point 4 μ m that can penetrate into the gas-exchange regions of the human lung [Second Pneumoconiosis Conference, Johannesburg 1959] was sampled with cyclones (Casella SIMPEDS, Casella London Ltd., London, UK) at flow rate 1.9 lpm. All dust samples were collected on membrane filters made of pure

cellulose acetate, pore-size 0.8 μm (MFS, Dublin, CA) that were weighed on a microbalance in a controlled weighing room ($R_f = 55\%$, $T = 21^\circ\text{C}$) before and after sampling. The amount of α -amylase on the membrane filters was determined by an immuno assay based method described elsewhere [Houba et al., 1996; Lillienberg et al., 2000].

BAL Procedure and Handling of Cells

Bronchoscopy with BAL was performed 2–6 weeks before the exposure and on the day after. The procedure for the bronchoscopy was performed as described in detail elsewhere [Eklund and Blaschke, 1986]. Shortly, after pre-medication with morphine and scopolamine, the bronchoscopy was carried out with a flexible fiberoptic bronchoscope (Olympus BF Type P20/30/40, Olympus Optical Co. Ltd, Tokyo, Japan) under local anesthesia. The bronchoscope was wedged in a subsegmental bronchus in the middle lobe and a total amount of 250 ml sterile phosphate-buffered saline (PBS) at 37°C was instilled in five aliquots of 50 ml each. After instillation, the fluid was gently aspirated and collected in a siliconized plastic bottle kept on ice. The BAL fluid was filtered through a single layer of Dacron nets (Type AP32, Millipore, Cork, Ireland) and centrifuged at 400g for 10 min at 4°C . The cells were resuspended in PBS and total cell count and determination of cell viability through trypan blue cell exclusion were performed using a Bürker chamber. Smears for differential counts were prepared by cytocentrifugation (Cytospin 2; Shandon, Runcorn, Cheshire, UK) at 22g for 3 min. After staining with May-Grünwald Giemsa, 500 cells were counted and after staining with toluidine/hematoxylin, the number of mast cells in ten visual fields ($16 \times$ magnification) was determined.

Venous blood samples were drawn at the time of bronchoscopy. Peripheral blood lymphocytes (PBL) were separated by Ficoll Hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation, washed twice, and diluted in PBS.

Immunofluorescence and Flow Cytometry

Immunolabelling of AM was carried out using primary unconjugated monoclonal mouse antibodies against adhesion molecules, co-stimulatory molecules, and markers for AM activation. Isotype-matched mouse immunoglobulin controls were used to compensate for non-specific background fluorescence (Table I). For each analysis, 5×10^5 cells were incubated for 30 min at 4°C in the dark with a saturating amount of primary antibody. After two washes with PBS the cells were stained with a secondary R-phycoerythrin (RPE)-conjugated $F(ab')_2$ rabbit antimouse IgG for 30 min. The cells were washed twice, resuspended in Cellfix (Becton Dickinson, Mountain View, CA), and kept on ice until flowcytometric analysis the following day. For analysis of T cell subpopulations and the expression of T cell activation markers, 2×10^5 cells were stained with a saturating amount of direct labeled monoclonal mouse antibodies. RPE-conjugated anti-CD8 antibodies and RPE-Cy5-conjugated anti-CD4 antibodies were simultaneously added together with FITC-conjugated anti-CD3, anti-CD69, anti-HLA-DR, or anti-CD45RA (Table I). To determine the number of natural killer (NK) cells, the lymphocytes were double stained with FITC-conjugated anti-CD3 and RPE-conjugated anti-CD56 antibodies. FITC-conjugated anti-TCR- $\gamma\delta$ -1 was used as a marker for the $\gamma\delta$ + T cell population (Table I). After incubation for 30 min at 4°C in the dark, the cells were washed twice in PBS, resuspended in Cellfix, and kept on ice over night before flowcytometric analysis.

Cells were analyzed in a FACSCalibur flowcytometer (Becton Dickinson). AM were gated by forward and side scatters and 10,000 cells were collected within the macrophage gate. Expression of each surface marker was determined as the mean fluorescence intensity (MFI) after subtraction of the background fluorescence of the control antibodies. Lymphocytes were also gated by their light scattering properties (10,000 cells were collected) and the percentage

TABLE I. Characterization of Monoclonal Antibodies (MoAb) Used for Staining of Lymphocytes (Ly) and Alveolar Macrophages (AM)

MoAb antihuman-	Major function/marker of	Clone	Source	Used for staining of
CD11a	Adhesion	MHM24	DAKO	AM
CD14	Monocyte marker	TÜK 4	DAKO	AM
CD45-RA	Naive T-cells	L48	Becton Dickinson	Ly
CD54	Adhesion	MCA1135	Serotec	AM
CD56	NK cell marker	MOC-1	DAKO	Ly
CD69	Activation	L78	Becton Dickinson	Ly
CD80	Co-stimulation	DAL-1	Serotec	AM
CD86	Co-stimulation	BU63	Serotec	AM
HLA-DP, -DQ, -DR	Activation	CR3/43	DAKO	AM + Ly
TCR- $\gamma\delta$ -1	$\gamma\delta$ T cell marker	11F2	Becton Dickinson	Ly

of positively labeled cells in the CD4+ and CD8+ subsets were calculated. In BAL fluid, the percentage of NK cells was calculated by dividing the number of CD3–/CD56+ cells with the sum of CD3+ and CD3–/CD56+ cells in order to avoid miscalculations due to contaminating erythrocytes. The gamma delta ($\gamma\delta$) T cells were expressed as the percentage of CD3+ cells. Isotype matched negative control antibodies stained less than 1% of the analyzed cells in all experiments.

Statistical Analysis

The data are presented as medians with interquartile ranges. Statistical comparisons were made using Wilcoxon matched pairs test for comparisons between before and after values from the same individual and the non-parametric Mann–Whitney *U*-test were used for comparisons between the study group exposed to wheat flour alone and the group receiving flour with α -amylase. For correlation analysis, the non-parametric Spearman correlation coefficient was determined and tested for statistical significance.

RESULTS

Dust Sampling Data

The dust concentrations in the chamber during the exposure are shown in Table II. The total dust concentration did not differ between the groups, but the concentration of respirable dust was slightly lower in the group that was exposed to wheat flour with α -amylase added, $P < 0.05$ (Table II). Inhalable dust was measured during exposure of five subjects and was found to be close to the total dust value (Table II). The concentration of α -amylase was measured in all dust samples. A small amount of α -amylase was detected in the original flour (0.1 mg/kg) but could not be detected in

any of the dust samples collected during the exposure to wheat flour alone. The concentration of α -amylase in the chamber during exposure to wheat flour with α -amylase added was very similar in the inhalable dust and total dust samples and is reported for the inhalable dust in Table II. No α -amylase could be detected in the respirable dust.

BAL Cell Count Data

Recovery and BAL cell viability was $>70\%$ and $>90\%$, respectively, and did not differ before and after exposure. Exposure to wheat flour significantly increased the total number of cells in BAL fluid (Table III, upper part). The proportion of BAL cell subsets was similar before and after exposure (Table III, upper part).

There was a significant difference in the dust-induced change of total BAL cell concentration between the study group exposed to wheat flour dust only ($n = 10$; increase with $91.9 \times 10^6/L$) and the group exposed to flour dust with the baking additive fungal α -amylase ($n = 5$; decrease with $5.4 \times 10^6/L$), ($P < 0.01$). Also the change in concentration of AM differed significantly between the two groups ($P < 0.01$), with an increase in the concentration of AM in the flour dust group ($75.2 \times 10^6/L$) and a decrease in the α -amylase group ($7.5 \times 10^6/L$). The changes in the other BAL cell populations did not differ between the study groups.

The increase in BAL cell concentration showed a positive correlation with the concentration of respirable dust in the exposure chamber ($P < 0.001$) (Fig. 1a). Also the concentration of alveolar macrophages (AM) correlated with the amount of respirable dust ($P < 0.001$) (Fig. 1b) while for lymphocytes there was a positive trend ($P = 0.07$) (Fig. 1c).

Flowcytometric Analysis

None of the flowcytometric parameters analyzed showed any difference between the two study groups, i.e., with and without α -amylase added. The flowcytometry data are therefore presented from all 15 individuals as one group.

Alveolar Macrophages

There was a significant increase in the expression of CD14 after exposure (Table III, lower part). The AM expression of the adhesion molecule CD11a, the co-stimulatory surface antigen CD86, and the activation marker HLA-DR all decreased significantly after exposure to wheat flour dust. The expression of CD54 and CD80 did not change after exposure (Table III, lower part).

Lymphocyte Activation

The expression of the activation markers CD69 and HLA-DR was significantly higher among the BAL CD4+

TABLE II. The Air Concentration of Dust and α -Amylase During Exposure With Wheat Flour Alone and Wheat Flour With α -Amylase Added

Concentration of	Study group	
	Wheat flour n = 10	Wheat flour + α -amylase n = 5
Total dust (mg/m ³)	4.7 (4.6–4.9)	4.9 (4.6–5.0)
Inhalable dust (mg/m ³) ^a	Not measured	5.8 (5.8–6.2)
Respirable dust (mg/m ³) ^b	0.30 (0.27–0.36)	0.23 (0.19–0.26) ^c
α -amylase in inhalable dust (ng/m ³)	Not detected	57.4 (40.2–60.4)

Data are shown as medians with upper and lower quartiles in parenthesis.

^aInhalable dust: particles with cut-point 100 μ m.

^bRespirable dust: particles with cut-point 4 μ m.

^c $P < 0.05$ compared to the group exposed to wheat flour alone.

TABLE III. Bronchoalveolar Lavage (BAL) Fluid Cell Count Data and Alveolar Macrophage Mean Fluorescence Intensity (MFI) Values From 15 Healthy Individuals Before and After 1-hr Exposure to Wheat Flour Dust

	Before ^a	After ^b	P-value
Total cell count ($\times 10^6$)	15.5 (13.4–18.7)	22.3 (15.6–31.3)	<0.01
Total cell concentration ($\times 10^6/L$)	75.4 (70.4–104.1)	127.4 (92.1–187.4)	<0.01
Macrophages (%)	92.8 (90.9–94.7)	93.0 (89.5–94.4)	ns
Lymphocytes (%)	6.4 (5.0–7.9)	5.0 (4.1–9.1)	ns
Neutrophils (%)	1.0 (0.7–1.5)	0.8 (0.5–2.8)	ns
Eosinophils (%)	0.2 (0.0–0.4)	0.0 (0.0–0.2)	ns
Basophils (%)	0.0 (0.0–0.0)	0.0 (0.0–0.2)	—
MAST cells ^c	4.0 (1.0–7.0)	4.0 (3.0–9.5)	ns
CD11a ^d (MFI)	204.8 (173.6–217.0)	66.4 (137.6–194.8)	<0.05
CD14 (MFI)	28.5 (22.7–38.9)	37.6 (31.9–53.9)	<0.001
CD54 (MFI)	44.0 (37.9–52.9)	43.4 (37.3–52.1)	ns
CD80 (MFI)	5.3 (3.7–7.7)	7.1 (5.1–10.7)	ns
CD86 (MFI)	51.9 (48.3–58.2)	47.9 (42.2–52.1)	<0.05
HLA-DR (MFI)	1,053.0 (958.6–1,266.0)	880.4 (764.9–1,070.0)	<0.05

Data are shown as medians with upper and lower quartiles in parenthesis.

^aTwo to six weeks before exposure.

^bOne day after exposure.

^cNumber in ten visual fields, $\times 16$ magnification.

^dn = 14.

and CD8+ T cells compared to the cells in peripheral blood ($P < 0.001$ for both markers in both CD4+ and CD8+ T cells). The expression of CD45-RA was significantly lower in BAL lymphocytes compared to PBL ($P < 0.001$ for both CD4+ and CD8+ T cells) (Table IV). The expression of CD69, HLA-DR, and CD45-RA on BAL CD4+ and CD8+ T cells did not show any significant changes after exposure to wheat flour dust. In peripheral blood on the other hand, there was a slightly increased expression of HLA-DR after exposure among both the CD4+ cells the CD8+ T cells (Table IV). The expression of CD69 was low among both CD4+ and CD8+ cells in peripheral blood and remained low also after exposure. No change was detected in the expression of CD45-RA in either T-cell subset in peripheral blood (Table IV).

Lymphocyte Subpopulations

In BAL fluid, the CD4+/CD8+ cell ratio was 2.3 (1.9–3.3) before and 2.0 (1.4–2.8) after exposure to wheat flour dust. The CD4+/CD8+ cell ratio in peripheral blood was similar compared to BAL fluid and remained unchanged after exposure. Table V shows the percentages of CD4+ and CD8+ T cells, NK cells (CD3–/CD56+), and $\gamma\delta$ T cells in BAL fluid and peripheral blood before and after exposure to wheat flour. The percentage of NK cells in peripheral blood

was significantly higher than in BAL fluid ($P < 0.001$, before and after exposure). The proportion of CD3+ cells expressing the $\gamma\delta$ T cell receptor was similar among lymphocytes from peripheral blood and BAL fluid. None of the lymphocyte subpopulations in peripheral blood or in BAL fluid changed significantly after exposure (Table V).

DISCUSSION

Airway inflammation due to organic dust in the working environment, e.g., in bakeries, is a common health problem. However, studies of cellular responses to inhalation of wheat flour dust have not previously been carried out. In this work, we investigated the effects of exposure to wheat flour dust on the cellular response in BAL fluid and peripheral blood of healthy individuals.

Our group has recently developed a whole-body exposure chamber for provocation studies with air-borne allergens and irritants, and it has been tested for exposure to wheat flour dust [Lidén et al., 1998]. The chamber offers a well-controlled exposure environment, with reproducible levels of exposure. A preliminary study showed that exposure of healthy individuals to wheat flour led to an influx of inflammatory cells into the bronchoalveolar space (unpublished data). Exposure to cornstarch, used to investigate the effects of the starch component of wheat flour, also

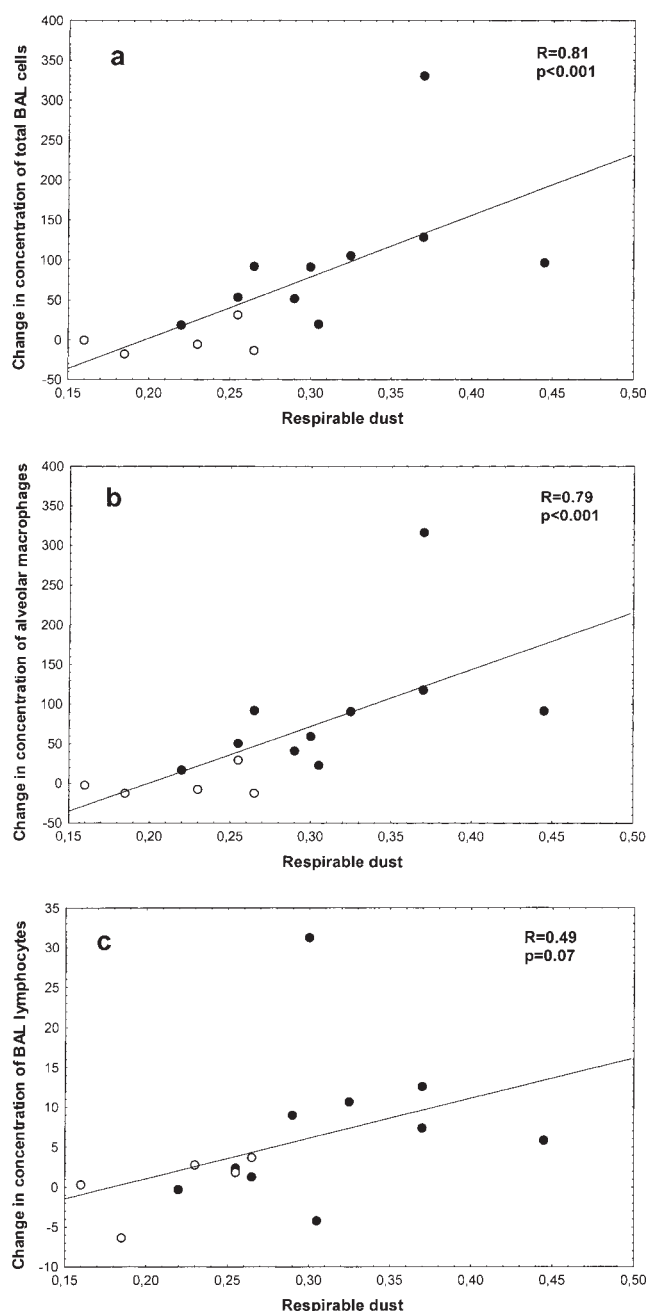


FIGURE 1. Relationship between the concentration of respirable wheat flour dust in the exposure chamber and the change in concentration (concentration after minus concentration before) ($\times 10^6/L$) of (a) total cells, (b) macrophages, and (c) lymphocytes in bronchoalveolar lavage (BAL) fluid. BAL from 15 healthy volunteers 2–6 weeks before and 1 day after 1-h exposure to wheat flour dust alone (filled circles, $n = 10$) or wheat flour dust with the flour additive fungal α -amylase (open circles, $n = 5$).

gave rise to an increased BAL cell concentration. In addition, cornstarch exposure resulted in a dramatic accumulation and activation of eosinophils in the airways of healthy individuals [Grunewald et al., 2003].

In the current investigation, we were interested in studying any effect on BAL fluid and peripheral blood cells following exposure of healthy individuals to wheat flour dust with or without fungal α -amylase. We found that healthy individuals exposed to flour dust responded with an increase in the total BAL cell concentration, although the proportion of the different cell types remained unchanged. Interestingly, in the group exposed to wheat flour with α -amylase added, we could not register any increase in BAL cell concentration at all. However, the level of respiratory dust during exposures was lower for wheat flour with α -amylase added compared to wheat flour alone. Although we have no explanation for this bias, it is likely that the mixing with α -amylase could cause some change in the wheat flour's properties. The magnitude of the cellular response in BAL fluid showed a positive correlation with the concentration of respirable dust. This might reflect the importance of the respirable fraction of the dust reaching the more distal parts of the airways, i.e., from where the lavage fluid is retrieved, to induce an increase in BAL cell concentration.

It has been shown that AM are an important source of the increased production of pro-inflammatory cytokines after exposure to corn dust [Becker et al., 1999].

In our study, analyses of AM in BAL fluid showed a significant increase in the expression of CD14 (LPS-receptor), while the activation marker HLA-DR, the co-stimulatory molecule CD86, and the adhesion surface molecule CD11a all decreased significantly after exposure to wheat flour. This is most likely a result of an influx of monocytic cells into the lungs similar to what we have reported following allergen provocation in asthmatic patients [Lensmar et al., 1999]. Monocytic cells migrating to the bronchoalveolar space have a higher expression of CD14 and are also less activated than the AM [Stent et al., 1995; Van Hal et al., 1995].

We also studied markers for lymphocyte subpopulations, i.e., CD4 (T-helper cells), CD8 (cytotoxic T cells), CD56 (NK cells), and $\gamma\delta$ TCR ($\gamma\delta$ T cells), and the expression of the early and late activation markers CD69 and HLA-DR respectively, in both peripheral blood and BAL fluid. There were no changes in the different subpopulations after exposure to wheat flour dust. NK cells were proportionally more frequent in PBL compared to BAL fluid, while we found similar levels of $\gamma\delta$ T cells in blood and BAL fluid.

As shown previously, there was a significantly higher expression of the CD69 and HLA-DR in BAL lymphocytes compared to PBL [Katchar et al., 2001]. While the expression of the activation markers in BAL lymphocytes remained unchanged after exposure, there was a tendency of the lymphocytes in peripheral blood to show an increased expression of the activation marker HLA-DR, suggesting that exposure to airborne antigens in a whole body chamber may influence the immune system systemically.

TABLE IV. T Cells in Peripheral Blood (PBL) and BAL Fluid Expressing the Markers CD69, HLA-DR, and CD45-RA

Marker	BAL				PBL			
	CD4+		CD8+		CD4+		CD8+	
	Before ^a	After ^b	Before ^a	After ^b	Before ^a	After ^b	Before ^a	After ^b
CD69	75.1 (63.5–78.6)	73.1 (60.0–78.9)	79.1 (78.1–85.3)	79.4 (77.3–85.4)	0.7 (0.5–0.9)	0.8 (0.8–1.3)	1.5 (1.1–2.2)	1.9 (1.4–2.4)
HLA-DR	40.6 (32.9–47.5)	42.6 (35.8–53.8)	32.4 (31.1–43.4)	33.5 (30.0–46.4)	5.8 (2.8–8.2)	6.7 (4.6–11.0) ^c	6.1 (3.7–12.0)	11.2 (7.1–17.8) ^d
CD45-RA	2.5 (2.2–4.5)	2.8 (1.9–7.0)	3.3 (2.1–5.6)	4.5 (3.6–6.5)	52.5 (46.4–59.6)	48.0 (44.1–58.4)	59.2 (46.6–69.2)	58.5 (51.6–63.5)

Data are presented for 15 individuals before and after 1-hr exposure to wheat flour dust. Data are shown as medians with upper and lower quartiles in parenthesis.

^aTwo to six weeks before exposure.

^bOne day after exposure.

^c $P < 0.01$ compared to the “before” value.

^d $P < 0.05$ compared to the “before” value.

TABLE V. Lymphocyte Subset Data

Subpopulation	BAL		PBL	
	Before ^a	After ^b	Before ^a	After ^b
CD4+	63.0 (60.6–69.7)	59.1 (53.6–72.6)	60.1 (52.5–66.5)	60.7 (53.3–67.7)
CD8+	27.6 (22.8–32.2)	28.8 (23.3–33.8)	30.8 (26.2–38.3)	30.7 (26.6–39.6)
$\gamma\delta$ T-cells	3.4 (2.2–5.9)	3.6 (3.6–6.1)	5.1 (3.7–7.7)	4.3 (3.4–7.1)
NK-cells	4.0 (3.7–6.6)	4.5 (3.6–6.5)	12.3 (10.6–19.2)	11.1 (9.1–18.0)

Median percentage of the different lymphocyte subpopulations in peripheral blood (PBL) and BAL. Data are shown as medians with upper and lower quartiles in parenthesis.

^aTwo to six weeks before exposure.

^bOne day after exposure.

In this and previous studies using the exposure chamber, we exposed healthy individuals to dust on a single occasion, while previous studies have concentrated on the effects of individuals with baker's asthma sensitized to wheat flour allergens [Górski et al., 2000]. Interestingly, one comparative study of non-atopic versus atopic subjects showed that atopy did not seem to play a major role in the development of grain-dust induced airway disease [Blaski et al., 1996a]. Since sensitization requires repeated exposures, our type of study would not reveal any allergic reactions to flour allergens. The influx of inflammatory cells into the airways more likely reflects a reaction to a non-specific irritative effect of the dust. Individuals exposed to grain dust showed an increased cellularity in nasal lavage [Blaski et al., 1996b]. Also, non-asthmatic, non-atopic grain handlers have been shown to develop a marked inflammatory response following exposure to corn dust [Clapp et al., 1994]. However, in these studies endotoxin may be responsible for the increased cellularity, which mainly consisted of neutrophils. In our study, the cellular composition of the BAL fluid might have

been different if we had done the analysis shortly after the exposure. Based on earlier studies in our group [Müller-Suur et al., 1997; Grunewald et al., 2003] we performed the bronchoscopy and the cellular analysis 20 hr after exposure. An interesting future study would be to use our whole-body exposure chamber to investigate the response to wheat flour dust in sensitized versus non-sensitized bakers.

In conclusion, we have used a whole-body exposure chamber to study the cellular response in healthy individuals exposed to either wheat flour dust alone or to flour dust together with the baking additive fungal α -amylase. There was a distinct increase in the total cell concentration in BAL fluid in the wheat flour group, while there was no significant change in the group where α -amylase was added. We also found a positive correlation between the concentration of respirable dust in the exposure chamber and the change in total cell concentration. The results indicate that the amount of respirable wheat flour dust, but not α -amylase, is of importance for the recruitment of inflammatory cells to the airways of healthy individuals.

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REFERENCES

- Baur X, Posch A. 1998. Characterized allergens causing bakers' asthma. *Allergy* 53:562–566.
- Baur X, Degens PO, Sander I. 1998. Baker's asthma: Still among the most frequent occupational respiratory disorders. *Allergy Clin Immunol* 102:984–997.
- Becker S, Clapp WA, Quay J, Frees KL, Koren HS, Schwartz DA. 1999. Compartmentalization of the inflammatory response to inhaled grain dust. *Am J Respir Crit Care Med* 160:1309–1318.
- Blaski CA, Clapp WD, Thorne PS, Quinn TJ, Watt JL, Frees KL, Yagla SJ, Schwartz DA. 1996a. The role of atopy in grain dust-induced airway disease. *Am J Respir Crit Care Med* 154:334–340.
- Blaski CA, Watts JL, Quinn TJ, Thorne PS, Schwartz DA. 1996b. Nasal lavage cellularity, grain dust, and airflow obstruction. *Chest* 109:1086–1092.
- Brisman J, Järholm B. 1995. Occurrence of self-reported asthma among Swedish bakers. *Scand J Work Environ Health* 21:487–493.
- Burdorf A, Lillienberg L, Brisman J. 1994. Characterization of exposure to inhalable flour dust in Swedish bakeries. *Ann Occup Hyg* 38:67–78.
- Clapp WD, Becker S, Quay J, Watt JL, Thorne PS, Frees KL, Zhang X, Koren HS, Lux CR, Schwartz DA. 1994. Grain dust-induced airflow obstruction and inflammation of the lower respiratory tract. *Am J Respir Crit Care Med* 150:611–617.
- Cullinan P, Lowson D, Nieuwenhuijsen MJ, Sandiford C, Tee RD, Venables KM, McDonald JC, Newman Taylor AJ. 1994. Work-related symptoms, sensitization, and estimated exposure in workers not previously exposed to flour. *Occup Environ Med* 51:579–583.
- Cullinan P, Cook A, Nieuwenhuijsen MJ, Sandiford C, Tee RD, Venables KM, McDonald JC, Newman Taylor AJ. 2001. Allergen and dust exposure as determinants of work-related symptoms and sensitization in a cohort of flour-exposed workers; a case-control analysis. *Ann Occup Hyg* 45:97–103.
- Eklund A, Blaschke E. 1986. Relationship between changed alveolar-capillary permeability and angiotensin converting enzyme activity in serum in sarcoidosis. *Thorax* 41:629–634.
- Grunewald J, Eklund A, Katchar K, Moshfegh A, Lidén C, Lundgren L, Skare L, Tornling G. 2003. Lung accumulations of eosinophil granulocytes after exposure to cornstarch glove powder. *Eur Respir J* 21:646–651.
- Górski P, Krakowiak A, Ruta U. 2000. Nasal and bronchial responses to flour-inhalation in subjects with occupationally induced allergy affecting the airway. *Int Arch Occup Environ Health* 73:488–497.
- Houba R, Heederik DJ, Doekes G, van Run PE. 1996. Exposure-sensitization relationship for alpha-amylase allergens in the baking industry. *Am J Respir Crit Care Med* 154:130–136.
- Karjalainen A, Keskinen H, Nordman H, Kurppa K. 1998. Incidence of reported occupational asthma in Finland 1990–1995. In: Chiyotani K, Hosoda Y, Aizawa Y, editors. *Advances in the prevention of occupational respiratory diseases*. Amsterdam: Elsevier. p 434–438.
- Katchar K, Wahlström J, Eklund A, Grunewald J. 2001. Highly activated T-cell receptor AV2S3+ CD4+ lung T-cell expansions in pulmonary sarcoidosis. *Am J Respir Crit Care Med* 163:1540–1545.
- Lenzmar C, Prieto J, Dahlen B, Eklund A, Grunewald J, Roquet A. 1999. Airway inflammation and altered alveolar macrophage phenotype pattern after repeated low-dose allergen exposure of atopic asthmatic subjects. *Clin Exp Allergy* 29:1632–1640.
- Lidén C, Lundgren L, Skare L, Lidén G, Tornling G, Krantz S. 1998. A new whole-body exposure chamber for human skin and lung challenge experiments—the generation of wheat flour aerosols. *Ann Occup Hyg* 42:541–547.
- Lillienberg L, Baur X, Doekes G, Belin L, Raulf-Heimsoth M, Sander I, Stahl A, Thissen J, Heederik D. 2000. Comparison of four methods to assess fungal alpha-amylase in flour dust. *Ann Occup Hyg* 44:427–433.
- McDonald JC, Keynes HL, Meredith SK. 2000. Reported incidence of occupational asthma in United Kingdom, 1989–1997. *Occup Environ Med* 57:823–829.
- Müller-Suur C, Larsson K, Malmberg P, Larsson PH. 1997. Increased number of activated lymphocytes in human lung following swine dust inhalation. *Eur Respir J* 10:376–380.
- Nieuwenhuijsen MJ, Heederick D, Doekes D, Venables KM, Newman Taylor AJ. 1999. Exposure-response relations of alpha-amylase sensitization in British bakeries and flour mills. *Occup Environ Med* 56:197–201.
- Second Pneumoconiosis Conference, Johannesburg 1959. 1960. Recommendations adopted by the pneumoconiosis conference. In: Orenstein AJ, editor. *Proceedings of the Pneumoconiosis Conference held at the University of Witwatersrand, Johannesburg, 9–24 February, 1959*. London, UK: J & A Churchill. p 617–621.
- Stent G, Irving L, Lewin S, Crowe SM. 1995. The kinetics of surface expression of CD11/CD18 integrins and CD54 on monocytes and macrophages. *Clin Exp Immunol* 100:366–376.
- Tikkanen U, Louhelainen K, Nordman H. 1996. The Nordic expert group for criteria documentation of health risks from chemicals: Flour dust. *Arbete Och Hälsa* 27:1–51.
- Van Hal PT, Wijkhuijs JM, Mulder PG, Hoogsteden HC. 1995. Proliferation of mature and immature subpopulations of bronchoalveolar monocytes/macrophages and peripheral blood monocytes. *Cell Prolif* 28:533–543.