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Endotoxin-binding proteins in nasal lavage: evaluation as biomarkers to occupational endotoxin exposure

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Exposure to endotoxin (LPS) can cause chronic respiratory disease, with symptoms that are more pronounced after exposure-free periods. The aim of this study was to evaluate LPS-response modulating proteins in nasal lavage and plasma as biomarkers for exposure to airborne endotoxin. We applied nasal lavage, lung function and exposure measurements in a small group ($n = 11$) of cotton workers during 6 weeks of observation (after 2 weeks free from exposure) and ten external controls. Lipopolysaccharide binding protein (LBP) and bactericidal/permeability increasing protein (BPI) were measured in nasal lavage fluid (NALF) along with classic markers such as differential cell counts, Interleukin-8 (IL-8) and albumin, to evaluate their use as markers in endotoxin exposure. In all control subjects and cotton workers LBP and BPI were readily detectable in NALF, although a high intra- and intervariability was noted. At the exposure levels in this study (cotton dust, geometric mean (GM) = 1.10 mg m^{-3}; endotoxin, GM = 2869 EU m^{-3}), plasma BPI and BPI and LBP in NALF were significantly ($P < 0.05$) different from external controls. In addition, within the group of cotton workers, during the measurement period a significant increase was noted in BPI, albumin and BPI/LBP ratio in NALF ($P < 0.05$), while a significant decrease in total cell numbers was noted. However, none of the markers in NALF was correlated to the different exposure indices used, based on personal endotoxin or dust exposure measurements. The data show that LBP and BPI are present in nasal lavage fluid and that these markers as well as their ratio increase during airborne endotoxin exposure in cotton workers.

Keywords: LPS, LBP, BPI, nasal lavage, cotton dust, endotoxin.

Introduction

Lipopolysaccharide (LPS), a glycolipid present in the cell membrane of Gram-negative bacteria, plays an important role in the airway response to inhaled organic dusts. The current understanding is that endotoxin, through release of proinflammatory cytokines, initiates an inflammatory response, causal in the genesis of airway reactivity and obstruction (Kunkel *et al.* 1994). Healthy subjects and asthmatics showed inflammation in bronchoalveolar lavage (Michel *et al.* 1992, 1997, Sandström *et al.* 1992), induced sputum (Nightingale *et al.* 1998) and blood (Michel *et al.* 1995, 1997) upon challenge with inhaled LPS between 20 and 25 μg. Typical workplaces known to contain high airborne amounts of LPS and leading to similar inflammatory responses are swine barns (Wang *et al.*1996) and grain processing plants (Blaski *et al.* 1996, Borm *et al.* 1996).

The response of the host is dependent on exposure but also supposed to be affected by the interaction between LPS and endogenous proteins. The acute phase

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reactant LPS-binding protein (LBP), which is present in plasma of healthy persons at levels of 10 μg ml^{-1}, is known to transfer LPS to CD14, thereby inducing macrophage activation (Wright *et al.* 1990). Expression of CD14 is found to be induced after *in vivo* and *in vitro* exposure to endotoxin and has therefore been forwarded as a marker of endotoxin exposure (Swan *et al.* 1998). LBP can also catalyse binding of LPS to lipoproteins, thus neutralizing the biological activity of LPS. Another important LPS-recognizing protein is bactericidal-permeability protein (BPI) which is present in the granules of polymorphonuclear leukocytes and on the surface of monocytes (Weiss *et al.* 1978, Dentener *et al.* 1997). Apart from its bactericidal activity, BPI strongly antagonizes LBP by binding and inactivation of LPS (Dentener *et al.* 1993). Both LBP and BPI have been measured in plasma of various patient groups and the ratio of BPI and LBP was forwarded as a sensitive marker with regard to infectious disease (Opal *et al.* 1994, Dentener *et al.* 1995, Froon *et al.* 1995). Previously, we measured LBP and BPI in plasma of grain workers and found a weak correlation between plasma LBP and individual cumulative exposure to inhalable grain dust (Borm *et al.* 1996) .

Here we report on the levels and response of these LPS-response modulating proteins in the nose as evaluated by nasal lavage, which is a convenient tool to study the inflammatory response of the upper respiratory tract (Graham and Koren, 1990, Persson *et al.* 1992). Nasal lavage studies among workers that are exposed to endotoxin through organic dusts such as grain dust, swine dust and wood dust have shown increased total cellularity, neutrophil concentrations, albumin, Interleukin-6 (IL-6) and Tumour Necrosis Factor-alpha (TNF) (Ahman *et al.* 1995, Blaski *et al.* 1996, Wang *et al.* 1996). The present study was done to investigate whether LBP and BPI are present in nasal secretions and plasma of cotton workers and whether they can be used as biomarkers to airborne endotoxin exposure.

Methods

Study design

Eleven workers exposed to cotton dust in a textile factory were included in a 6 week short-longitudinal study after a 2 week exposure-free period (figure 1). Written informed consent was obtained from each worker after evaluation of a validated questionnaire on respiratory symptoms, smoking and working history. Nasal lavage was performed post-shift (13.00–14.00 h) on Monday, Wednesday, and Friday during weeks 1 and 6, and analysed for inflammatory markers and total cells. Daily (8 h–time weighted average) personal exposure to airborne cotton dust was measured during weeks 1 and 6 of the observation period. All specimens were transported to our laboratory within 4 h of sampling for further processing (centrifugation, fixation).

Since it was impossible to obtain exposure-free controls from the textile-plant (others were exposed to bleach, paints, etc.), a control group of 11 external references working in our university research laboratory was investigated during the same time period and sampled during weeks 1, 3 and 6, also on Mondays, Wednesdays and Fridays. In the course of investigations one control subject (number 7) was excluded because of asthmatic symptoms. Endotoxin exposure for these individuals was measured incidentally during occupational surveillance checks and considered to be negligible (< 10 ng m^{-3}).

Exposure measurements

Personal inhalable dust exposure was measured daily in weeks 1 and 6 in 11 workers, using portable airpumps (Dupont, P-2500) and PAS-6 samplers at a sampling rate of 2.0 l min^{-1}. Total cotton dust concentrations were determined gravimetrically and endotoxin content of the dust was determined using the Limulus Amoebocyte Lysate (LAL)-test. Details of endotoxin extraction methods and analyses are given elsewhere (Douwes *et al.* 1995, Keman *et al.* 1998).

Nasal lavage and blood sampling

Nasal lavage (NAL) was performed as described previously (Keman *et al.* 1998). Briefly, the subject, in sitting position, lifted his neck approximately 45 degrees backward and elevated the palate to close his

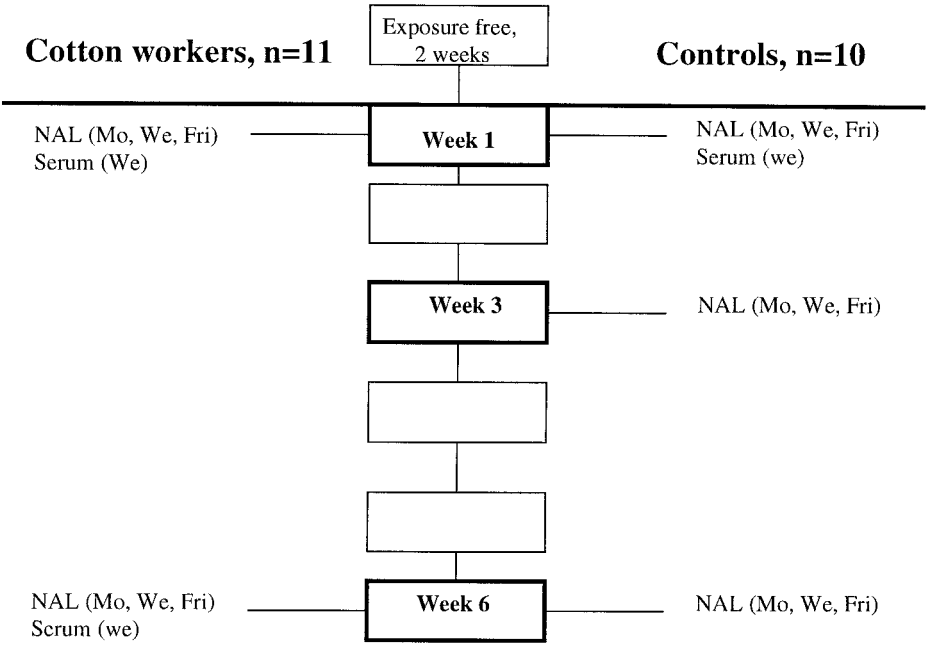

Figure 1. Graphic illustration of the short longitudinal study among exposed cotton dust workers ($n = 11$) and controls ($n = 10$). Nasal lavage in cotton workers was performed on Monday, Wednesday, and Friday afternoon during weeks 1 and 6; in control subjects nasal lavage was done at the same time-points in weeks 1, 3 and 6. During weeks 1 and 6, daily personal exposure to (airborne) total cotton dust levels were measured by gravimetric measurement.

nasopharynx. Five ml of lukewarm sterile phosphate buffered saline solution (Ca^{2+}- and Mg^{2+}-free) was instilled into each nostril using a 10 ml sterile polystyrene pipette, while the subject did not breath or swallow. After 10 s, the subject bent forward and the nasal lavage fluid was expelled into a 15 ml centrifuge tube via a polyamide gauze filtered funnel to separate mucus. The fluid was immediately put on ice, transported within 4 h to the laboratory and subsequently centrifuged at 600 *g* for 10 min. The supernatant was separated into eight aliquots (500 µl) and immediately stored at –70 °C until further analysis. Blood samples (10 ml) were drawn midweek only in EDTA-coated vacuum tubes, stored at 4 °C until transport to our laboratory and plasma was obtained by double centrifugation at 3000 *g*, avoiding aspiration of buffy coat cells. Plasma was frozen at –70 °C until determination of LBP and BPI.

NALF markers and cell counts

LBP and BPI were measured by modified sandwich ELISA techniques as described previously (Dentener *et al.* 1995, Froon *et al.* 1995, Borm *et al.* 1996, Keman *et al.* 1998). Albumin concentration was determined by turbidimetry (ARRAY, standard kits of Beckmann) in the Clinical Analytic Laboratory of the Maastricht Academic Hospital. Detection limits of the assays were 200 pg ml^{-1} (LBP), 100 pg ml^{-1} (BPI) and 2 mg l^{-1} (albumin). Fifty µl of the cell pellet after centrifugation of the NAL (500 µl) was used to determine the number of cells after formalin (50 µl, 1.1 %) fixation and calculated as the number of total cells per lavage as well as the number of cells per ml of recovered fluid. No detectable levels of TNFα were found in nasal lavage fluid (NALF) using ELISA assays (Borm *et al.* 1996) and therefore not further elaborated. The soluble 75 kDa TNF receptor was only detected in some subjects and only in few repeated measurements (Keman *et al.* 1998)

Statistical analysis

All results are expressed as median (25th–75th percentiles), unless otherwise stated. Paired and unpaired comparison and correlation of inflammatory markers in NAL were made between weeks 1 and 6 of observation using Wilcoxon matched-pairs signed-rank, Mann Whitney *U* and Spearman rank correlation tests, chosen for reason of group size and data distribution. For cotton workers the week average of each individual was used as a mean of three different nasal lavages, for controls the average of

Table 1. Demographic and health characteristics of the study groups.

Parameter	Controls	Cotton workers
General		
Number	10	11
Age (years)	24.7 ± 2.1	46 ± 5.2
Smoking (yes/no)	3/7	4/7
Years of exposure	none	25 (7–39)
Male/female	8/2	11/0
No. of nasal lavages	9	6
Respiratory symptoms (yes/no)		
Chronic bronchitis	0/10	1/10
Asthma	0/10	0/11
Medication	1/10	1/10
Exposure—cotton dust (mg m^{-3}) (GM ± GSD)		
Week 1	Not determined	1.35 ± 3.60 (n = 22)
Week 6	Not determined	1.30 ± 3.10 (n = 29)
Exposure—endotoxin (EU m^{-3}) (GM ± GSD)		
Week 1	Not determined	1541 ± 7 (n = 27)
Week 6	Not determined	3828 ± 3 (n = 34)

Not determined are considered to be < 10 ng\m^{-3}

three weeks was used. Comparisons between controls and cotton workers were done using all separate measurements as independent measures. Geometric standard deviations of inflammatory markers in NAL were calculated from mean squares obtained by ANOVA on log-transformed data as decribed by Boleij *et al.* (1995). All statistical analyses were done using Statistica 5.0 for Windows (Statsoft Inc., Tulsa, USA).

Results

Exposure and population characteristics

The average age of the cotton workers was 46 years and was significantly higher than the age of the controls (24.7 years). The control group contained two female subjects, but previous work has not revealed any differences in nasal response between males and females (Schins *et al.* 1997). Smoking was not significantly different between cotton workers and controls, but was included in further statistical analysis. The years of employment varied largely (7–39 years) among cotton workers and one out of 11 workers had symptoms of chronic bronchitis, while another worker used medication for nasal symptoms (table 1). One control (subject 7) was excluded because of asthmatic symptoms during the survey period; no asthmatic symptoms were observed among workers. Six workers completed all nasal lavage tests both in weeks 1 and 6, while from eight controls a full set (nine measurements in 3 weeks) of nasal lavages was obtained.

Airborne cotton dust concentrations (n = 51) and endotoxin levels (n = 61) measured by personal air sampling of cotton workers were best described by a lognormal distribution (K-S Lilliefors, both $P > 0.20$). The geometric mean (GM) of airborne cotton dust concentrations (1.32 mg m^{-3}) was moderate, but endotoxin levels (GM = 2566 EU m^{-3}) were quite high. No statistically significant difference in cotton dust and endotoxin levels was found between weeks 1 and 6 (table 1).

Levels of markers in NALF and plasma—controls

LBP and BPI were detectable in all control subjects. In 83 samples analysed in 10 subjects over 3 weeks, 76 samples contained detectable amounts of LBP and

Table 2. Biomarkers and their intra- and inter-subject variation in nasal lavage and plasma of control and cotton workers over the entire study period. Values are median and (25th–75th) percentiles of all measurements.

	Controls			Cotton workers		
Parameter	Median (25th–75th percentile)	GSD_{intra}	GSD_{inter}	Median (25th–75th percentile)	GSD_{intra}	GSD_{inter}
Nasal lavage						
Cells	18 (5.6–42.3)	ND	ND	0.004 (0.0–0.4) **	ND	ND
Albumin (mg l^{-1})	13.3 (5.5–26.3)	1.66	2.15	6.04 (3.13–11.1)*	1.6	2.44
LBP (ng ml^{-1})	2.3 (1.62–5.0)	1.89	1.97	1.03 (0.38–2.37)**	1.94	3.38
BPI (pg ml^{-1})	808 (398–1791)	2.04	2.59	455 (39–1395) *	4.01	4.04
Ratio BPI/LBP[a]	0.29 (0.12–0.60)	2.1	1.97	0.30 (0.09–0.78)	3.16	2.34
IL-8 (pg ml^{-1})	98 (27–391)	2.12	3.52	756 (483–2366) **	2.19	1.71
Plasma						
LBP (ng ml^{-1})	0.039 (0.025–0.046)	(b)	(b)	0.029 (0.017–0.037)	1.57	< 1.57
BPI (pg ml^{-1})	396 (205–486)	(b)	(b)	1890 (876–2593) *	1.69	1.53
Ratio BPI/LBP	0.005 (0.005–0.01)	(b)	(b)	0.06 (0.02–0.1) *	2.18	< 2.18

a) Significantly different from controls, *: $P < 0.05$, **: $P < 0.01$ (Wilcoxon-test); b) Ratio calculated with BPI and LBP in the same units, b) Only one plasma sample was taken in controls, not allowing ANOVA to calculate GSDs. ND, Not determined.

Geometric standard deviations for intra-subject variation (GSD_{intra}) and intersubject variation (GSD_{inter}) were calculated from an ANOVA on log-transformed data using the mean square of error (MS_{error}) as an estimate of intrasubject-variance (s^2_{intra}) and $MS_{between}$ as estimate of $\sigma^2_{intra} + n.\sigma^2_{inter}$, in which n is the number of repeated measurements per subject.

BPI. No significant differences in levels of LBP, BPI and albumin were present between the 3 weeks of measurement, but variability expressed as geometric standard deviations within and between the 10 control subjects was high (table 2). When expressed in variation coefficients average between subject-variability was 63, 74, and 45 % for LBP, BPI and albumin, respectively. Variation within subjects was in the same order of magnitude and was 112, 109, and 84 % for LBP, BPI and albumin. Part of the variation may be explained by smoking since both LBP and BPI were significantly lower in smokers compared with non-smokers ($P<0.05$) Although high, these variation coefficients are not different from other markers such as IL-8, IL-6, and cellularity reported on previously (Steerenberg *et al.* 1996, Keman *et al.* 1998).

As expected, levels of BPI in NALF were significantly correlated to total cell number and the percentage of PMN in nasal lavage (PMN: Spearman rank, $r = 0.42$, $P<0.01$). LBP levels were not correlated to any cell type or number present in nasal lavage but levels of BPI and LBP in NALF were significantly correlated to each other (Spearman rank, $r = 0.49$, $P<0.001$, $n = 76$). No relationship was present between NALF and plasma levels of biomarkers. Plasma levels of LBP were considerably higher than in nasal lavage. On the other hand plasma BPI and the median BPI/LBP ratio were lower than in NALF (table 2).

Levels of markers in NALF and plasma—cotton workers

LBP and BPI were readily detectable in most (> 80 %) nasal lavage samples of cotton workers (total samples, $n = 47$). Also in cotton workers, concentrations for LBP and BPI were lower in NALF than in plasma. When comparing NALF markers and cellularity between week 1 and week 6 after the exposure-free period

Table 3. Biomarkers in nasal lavage and plasma of all cotton workers measured in week 1 and week 6. Values are median and (25th–75th) percentiles of all measurements.

Parameter	Week 1 (n=24)	Week 6 (n=27)
NAL markers		
Cells ($\times 10^3$)	0.4 (0–1.1)	0.001 (0–0.001)
Albumin (mg ml^{-1})	3.8 (2.4–7.3)	8.9 (5.6–17.7) **
LBP (pg ml^{-1})	597 (394–1559)	1742 (252–2952)
BPI (pg ml^{-1})	245 (0–519)	1030 (172–3001) **
Ratio BPI/LBP	0.20 (0–0.49)	0.43 (0.22–1.62)*
IL-8 (pg ml^{-1})	726 (505–1006)	943 (460–1584)
Plasma markers		
LBP (μg ml^{-1})	25.5 (14.5–33.8)	29 (18–41)
BPI (pg ml^{-1})	1937 (860–2769)	1372 (808–2430)
Ratio BPI/LBP	0.07 (0.05–0.1)	0.05 (0.02–0.06)

Significantly different from data in week 1 (*, $P < 0.05$ and **$P < 0.01$, Wilcoxon-test). All separate measurements of each subject were used to do statistical testing.

(table 3) a significant decrease was observed in total number of cells and a significant increase in albumin, IL-8, BPI and the NALF BPI/LBP ratio. Other NALF and plasma markers were not changed during the 6 weeks including the average recovery of fluid (7.4 ml in week 1, and 6.6 ml in week 6). In the subgroup that completed both week 1 and week 6 only BPI, the BPI/LBP ratio and the total number of cells were significantly different (figure 2). No significant effect of smoking on NALF LBP and BPI was observed, which is probably explained by the smaller number of measurements and the confounding effect of exposure to LPS. Since nasal LBP (but not BPI) is expected to arise from increased plasma exudation and therefore might be related to increased albumin levels in NALF, LBP levels were corrected for albumin levels. As suggested the difference between controls and cotton workers (table 2) were no longer significant ($P = 0.074$, Wilcoxon), but on the other hand the difference between week 1 and week 6 ($P = 0.756$, NS) changed to $P = 0.19$ after correction. Although not biologically relevant, correction of IL-8 and BPI for albumin did not change the significance of most differences reported (tables 2 and 3). However, the highly significant difference in nasal lavage BPI between controls and cotton workers ($P = 0.029$) was lost after 'correction' ($P = 0.963$) for albumin.

Evaluation as exposure markers

Plasma concentrations of BPI were significantly higher in cotton workers in both weeks as compared with the average concentration in controls. No significant difference was observed between LBP concentrations in controls and cotton workers. Similar to findings in controls the BPI/LBP ratio in NALF fluid of cotton workers was higher compared with the plasma ratio (table 2). Only the ratio in NALF, however, increased significantly during exposure (table 3). To investigate a possible relation with cotton dust and/or endotoxin exposure, levels of LBP and BPI were correlated (Spearman Rank) to three personal exposure indices, i.e (i) exposure at the same day as the NAL, (ii) cumulative exposure in the days preceeding the NAL sample, and (iii) the cumulative week exposure. However, no significant correlations were found between the NAL markers in this study and any of these three exposure indices.

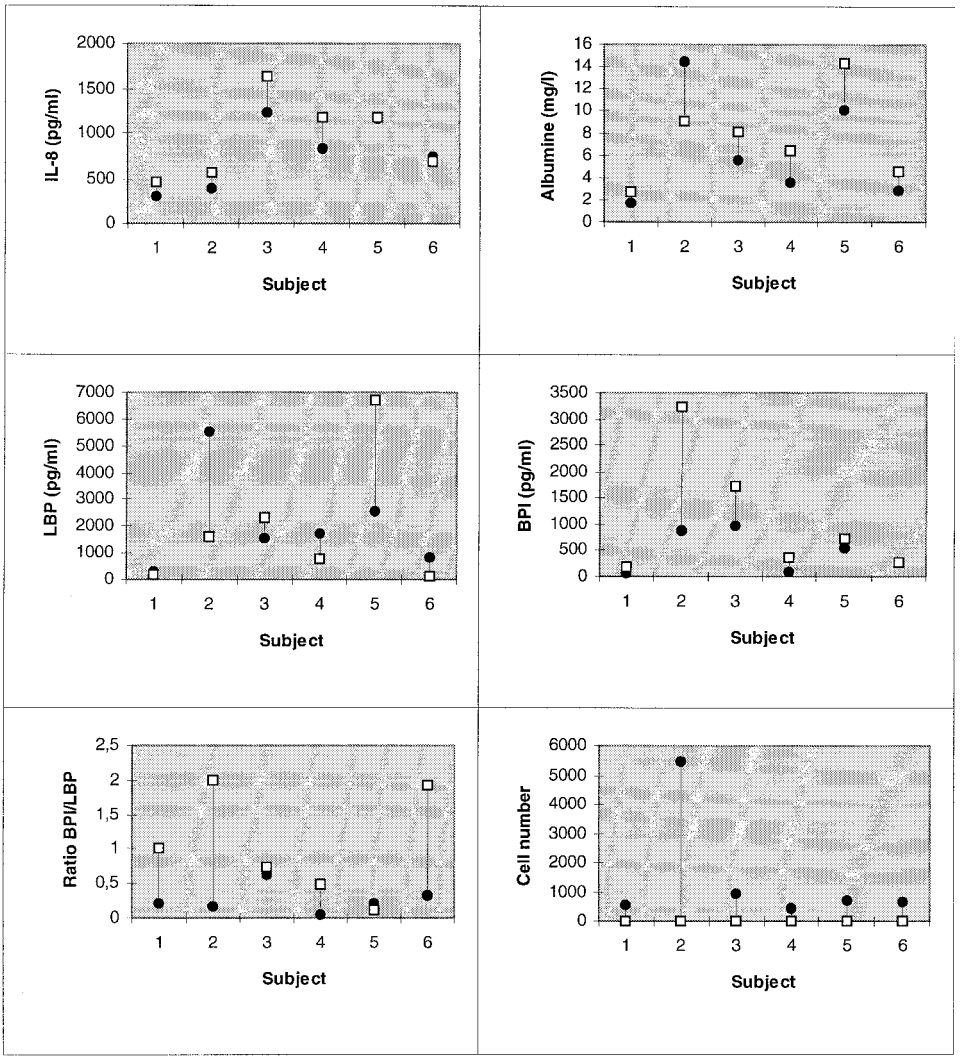

Figure 2. Graph illustrating the individual changes in IL-8, albumin, LBP, BPI , BPI/LBP ratio and cells in nasal lavage of the six individuals that were measured both in week 1 (v) and week 6 (M).

Discussion

In this study we applied nasal lavage and exposure measurements in a small group of cotton workers and control subjects during a period of 6 weeks. The aim of this study was to evaluate the presence and changes of LPS-complexing proteins and inflammatory markers in relation to endotoxin exposure after an exposure-free period. The reason to start after an exposure-free period was that usually respiratory symptoms, cross-shift lung function decline and fever are more pronounced, after which some adaptation seems to occur (Rylander 1990). Interestingly, LBP and BPI are present in nasal lavage fluid of both cotton workers and controls. Comparisons between week 1 and week 6 after start of exposure showed a consistent increase of BPI and BPI/LBP ratio in NALF. No significant longitudinal changes were observed in plasma levels of BPI and LBP.

Absolute concentrations of LBP in NALF were much lower than in plasma LBP. BPI levels on the other hand were in the same order of magnitude as in plasma and somewhat lower than in BAL fluid and pleural fluid of patients suspected from pneumonia or pleural effusion (Dentener *et al.* 1995). In contrast to the latter study, BPI in plasma was detectable in controls ($n = 10$, 76 out of 83 samples) and cotton workers. It has been suggested that mild PMN activation in blood can be the cause of conflicting data on this subject (White *et al.* 1994, Dentener *et al.* 1995). In this study EDTA-treated blood was kept for 4 h at 4 °C before transport and centrifugation, which might have caused this effect. Perhaps this also partly explains why plasma BPI in cotton workers was increased four-fold over controls, while no difference for LBP was seen. However, an effect of endotoxin exposure on plasma BPI also seems reasonable since previously we demonstrated *in vivo* priming of LPS on peripheral blood cells (Borm *et al.* 1996). Reference data for LBP, BPI in nasal lavage fluid are lacking but the values and mean coefficients of variation for the other markers are in the same range as other markers in nasal lavage (Steerenberg *et al.* 1996, Keman *et al.* 1998).The fact that BPI is mainly produced by neutrophils is in line with the observation that NALF-BPI levels were significantly correlated to total cells and percentage of PMN in control subjects and cotton workers. BPI is also correlated to IL-8 in NALF, but TNFα, known to be potent activator of BPI-release in whole blood and isolated PMN (Dentener *et al.* 1997), was not detectable in nasal lavage fluid (data not shown). Although monocytes have been demonstrated to express BPI on their cell surface (Dentener *et al.* 1996), the percentage of monocytes (< 2 %) is very low in NAL and not correlated to the NALF-BPI levels.

LBP is mainly synthesized in the liver, although recently it was reported that an alveolar type II cell-line also expresses and releases LBP (Dentener *et al.* 1999). That LBP in NALF is primarily the result of plasma exudation is supported by its correlation with NALF–albumin in cotton workers and controls, and the fact that differences between controls and cotton workers are lost after correction of LBP for albumin levels in NALF. It has to be noted however that NALF-BPI levels (and IL-8) were also correlated significantly to NALF–albumin in both groups, although their release is suggested to occur locally from PMN (BPI) and nasal epithelium (IL-8). In fact all these markers might be affected through a different effect of LPS inducing a transient increase in airspace epithelial permeability, leading to influx of PMN, albumin and plasma proteins (Li *et al.* 1998).

Both LBP and BPI tended to increase in NALF with ongoing exposure to endotoxin, although BPI showed the most pronounced and significant rise. Although not significant, the increase in NALF-LBP is somewhat suprising since LBP is known to enhance LPS toxicity and to antagonize the effects of BPI when binding to the CD14 receptor in macrophages. On the other hand LBP-binding was shown to inhibit cytokine production by monocytes incubated with PM2.5 suspensions containing LPS (Monn and Becker 1999). The ratio of both antagonizing proteins was also used to evaluate concomitant changes in BPI and LBP. Interestingly, the BPI/LBP ratio in nasal lavage is higher than in plasma of both controls and cotton workers. This might be explained by release of BPI from activated PMN in the nose. No data are available on the nasal lavage ratio but plasma ratios are well within range of controls as described in previous studies (Opal *et al.* 1994, White *et al.* 1994, Borm *et al.* 1996). In the plasma of cotton workers a slight, non-significant change of the ratio in week 6 was noted in plasma. However in the

nasal lavage a significant increase was noted. Previously, we studied the plasma ratio in workers exposed to grain dust and did not find a difference with controls. This could be explained by the 20-fold lower endotoxin exposure of the grain workers (GM < 10 ng m^{-3}) compared with these cotton workers (GM: 230 ng m^{-3}). However, these data also illustrate that the BPI/LBP ratio, when measured in nasal fluid, is a more sensitive marker to exposure of inhaled endotoxin than in plasma.

Suprisingly, the total number of cells decreased significantly throughout the measurement period. Most studies have reported increased NAL cells in subjects exposed to organic dusts (Ahman *et al.* 1995, Blaski *et al.* 1996, Wang *et al.* 1996) or other inflammatory agents like ozone (Graham and Koren 1990). Several methodological issues could contribute to our results, including nasal obstruction and an effect of exposure on mucous viscosity both affecting cell recovery. Most likely however, the decrease of cell number in week 6 is due to activation of PMN by continuous high LPS exposure (median: >2800 EU m^{-3} analogous to 250 ng m^{-3}) in combination with late (< 4 h after lavage) fixation. This may have resulted in cell death, lysis and release of BPI before further processing. One might imagine that leakage of BPI from activated PMN provides local protection against bacterial infections in the nose, although it is unclear whether BPI in biological fluids contains bactericidal capacity (Dentener *et al.* 1995). The high BPI/LBP ratio in the nose compared with plasma supports this concept.

In conclusion, our data show that endotoxin-response mediating proteins LBP and BPI are present in nasal lavage fluid of controls and that NALF but not plasma levels respond to exposure to endotoxin after an exposure-free period in cotton workers. However, the absence of a relation between NALF levels of LBP and BPI and personal exposure to inhalable dust and/or its endotoxin content, limits their value as possible exposure markers to endotoxin. However, when compared to other commonly used markers in NALF such as IL-8 and number of PMN and albumin, these proteins are very specific and sensitive markers. Since NAL can be much more easily obtained than blood or sputum at the workplace or in environmental screening, studies are presented that compare the non-response threshold of PMN-counts, plasma C-reactive-protein (Michel *et al.* 1997) or monocyte CD-14 expression (Swan *et al.* 1998) with the nasal response of LBP and BPI after acute inhalation of LPS or environmental particles containing LPS (Monn and Becker 1999). It remains to be determined whether the NALF response is related to individual differences in respiratory effects induced by endotoxin.

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References

AHMAN, M., HOLMSTRÖM, M. and INGELMAN-SUNDBERG, H. 1995, Inflammatory markers in nasal lavage fluid from industrial arts teachers. *American Journal of Industrial Medicine*, **28**, 541–550.

BLASKI, C. A., WATT, J. L., QUINN, T. J., THORNE, P. S. and SCHWARTZ, D. A. 1996, Nasal cellularity, grain dust and airflow obstruction. *Chest*, **109**, 1086–1092.

BOLEIJ, J., BURINGH, E., HEEDERICK, D. and KROMHOUT, H. 1995, *Occupational Hygiene of Chemical and Biological Agents* (Amsterdam: Elsevier Science BV), pp. 97–136.

BORM, P. J. A., SCHINS, R. P. F., DERHAAG, T. J. J. M. and KANT, I. J. 1996, Cross-shift changes in blood inflammatory markers occur in the absence of airway obstruction in workers exposed to grain dust. *Chest*, **109**, 1078–1083.

DENTENER, M. A., VON ASMUTH, E. J. U., FRANCOT, J. M., MARRA, M. N. and BUURMAN, W. A. 1993, Antagonistic effects of lipopolysaccharide protein and bactericidal/permeability protein on lipopolysaccharide-induced cytokine release by mononuclear phagocytes. *Journal of Immunology*, **151**, 4258–4265.

DENTENER, M. A., FRANCOT, G. J. M., SMIT, F. T., FROON, A. H. M., PENNINGS, H.-J., WOUTERS, E. F. M. and BUURMAN, W. A. 1995, Presence of bactericidal/permeability-increasing protein in disease detection by ELISA. *Journal of Infectious Diseases*, **171**, 739–743.

DENTENER, M. A., FRANCOT, G. J. M. and BUURMAN, W. A. 1996, Bactericidal/permeability-increasing protein, a lipopolysaccharide-specific protein on the surface of human peripheral blood monocytes. *Journal of Infectious Diseases*, **173**, 252–255.

DENTENER, M. A., FRANCOT, G. J. M., HIEMSTRA, P. S., TOOL, A. T. J., VERHOEVEN, A. J., VANDENABEELE, P. and BUURMAN, W. A., 1997, Bactericidal/permeability increasing protein release in whole blood ex-vivo: strong induction by lipopolysaccharide and tumor necrosis factor-α. *Journal of Infectious Diseases*, **175**, 108–117.

DENTENER, M. A., VREUGDENHIL, A. C. E., VERNOOY, J. H. J., JANSSEN, Y. M. W., BUURMAN, W. A. and WOUTERS, E. F. M. 1999, Production of the acute phase protein LPS binding protein (LBP) by alveolar type II cells. *American Journal of Critical Care Medicine*, **159**, A179.

DOUWES, J., VERSLOOT, P., HOLLANDER, A., HEEDERIK, D. and DOEKES, G. 1995, The influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Applied and Environmental Microbiology*, **61**, 1763–1769.

FROON, A. H. M., DENTENER, M. A., GREVE, J. W. M., RAMSAY, G. and BUURMAN, W. A. 1995, Lipopolysaccharide toxicity-regulating proteins in bacteremia. *Journal of Infectious Diseases*, **171**, 1250–1257.

GRAHAM, D. E. and KOREN, H. S. 1990, Biomarkers of inflammation in ozone-exposed humans: comparison of the nasal and bronchoalveolar lavage. *American Review of Respiratory Disease*, **142**, 152–156.

KEMAN, S., JETTEN, M., DOUWES, J. and BORM, P. J. A. 1998, Longitudinal changes of inflammatory markers in nasal lavage of cotton workers. *International Archives of Occupational and Environmental Health*, **71**, 131–137.

KUNKEL, S. L., CHENSUE, S. W., STANDIFORD, TJ. and STRIETER, R. M. 1994, Endotoxin-dependent cytokine networks. In *Endotoxin and the Lungs*, K. L. Brigham, ed. (New York: Marcel Dekker), pp. 305–320.

LI, X. Y., DONALDSON, K. and MACNEE, W. 1998. Lipopolysaccharide-induced alveolar epithelial permeability: the role of nitric oxide. *American Journal of Respiratory and Critical Care Medicine*, **157**, 1027–1033

MICHEL, O., GINANNI, R., LEBON, B., CONTENT, J., DUCHATEAU, J. and SERGYSELS, R. 1992, Inflammatory response to acute inhalation of endotoxin in asthmatic patients. *American Review of Respiratory Disease*, **146**, 352–357.

MICHEL, O., DUCHATEAU, J., PLAT, G., CANTINIEAUX, B., HOTIMSKY, A., GERAIN, J. and SERGEYSELS, R. 1995, Blood inflammatory response to inhaled endotoxin in normal subjects. *Clinical and Experimental Allergy*, **25**, 73–79.

MICHEL, O., NAGY, A. M., SCHROEVEN, M., DUCHATEAU, J., NEVE, J., FONDU, P. and SERGEYSELS, R. 1997, Dose–response relationship to inhaled endotoxin in normal subjects. *American Journal of Critical Care Medicine*, **156**, 1157–1164.

MONN, C. and BECKER, S. 1999, Cytotoxicity and induction of proinflammatory cytokines from human monocytes exposed to fine (PM2.5) and coarse particles (PM10-2.5) in outdoor and indoor air. *Toxicology and Applied Pharmacology*, **155**, 245–252.

NIGHTINGALE, J. A., ROGERS, D. F., HART, L. A., KHARITONOV, S. A., CHUNG, K. F. and BARNES, P. J. 1998, Effect of inhaled endotoxin on induced sputum in normal, atopic, and atopic asthmatic subjects. *Thorax*, **53**, 563–571.

OPAL, S. M., PALARDY, J. E., MARRA, M. N., FISHER, C. J. JR, MCKELLIGON, B. M. and SCOTT, R. W. 1994, Relative concentrations of endotoxin-binding proteins in body fluids during infection. *Lancet*, **344**, 429–431.

PERSSON, C. G. A., SVENSSON, C., GREIFF, L., ANDERSON, M., WOLLMER, P., ALKNER, U. and

ERJEVALT, I. 1992, The use of the nose to study inflammatory response of the respiratory tract. *Thorax*, **47**, 993–1000.

RYLANDER, R. 1990, Health effects of cotton dust exposure. *American Journal of Industrial Medicine*, **17**, 39–45.

SANDSTRÖM, T., BJERMER, L. and RYLANDER, R. 1992, Lipopolysaccharide (LPS) inhalation in healthy subjects increases neutrophyls, lymphocytes, and fibronectin levels in bronchoalve-olar lavage fluid. *European Respiratory Journal*, **5**, 992–996.

SCHINS, R. P. F., KNAAPEN, A., JETTEN, M. A. and BORM, P. J. A. 1997, Cell counts and inflammatory mediators in nasal lavage evaluated in two protocols using a cross-over design: a wash-out effect? *European Respiratory Journal*, **10** (suppl 25), 257S.

STEERENBERG, P. A., FISCHER, P. H., MEYLING, F. G., WILLIGHAGEN, J., GEERSE, E., VD VLIET, H., AMELING, C., BOINK, A. B. T. J., DORMANS, J., VAN BREE, L. and VAN LOVEREN, H. V. 1996, Nasal lavage as a tool for health effect of photochemical air pollution. *Human and Experimental Toxicology*, **15**,111–119.

SWAN, J. R. M., CURRAN, A. D. and BECKETT, P. N. 1998, The potential of a monocyte cell surface marker as indicator of endotoxin exposure. *Biomarkers*, **3**, 73–79.

WANG, Z., MALMBERG, P., LARSSON, P., LARSSON, B. M., and LARSSON, K. 1996, Time course of Interleukin-6 and Tumor Necrosis Factor-α increase in serum following inhalation of swine dust. *American Journal of Respiratory and Critical Care Medicine*, **153**,147–152.

WEISS, J., ELSBACH, P., OLSSON, I. and ODEBERG, H. 1978, Purification and characterization of a potent bactericidal and mebrane active protein from the granules of human polymorhonuclear leukocytes. *Journal of Biological Chemistry*, **253**, 2664–2672.

WHITE, M. L., MA, J. K., BIRR, C. A., TROWN, P. W. and CARROLL, S. F. 1994, Measurements of bactericidal/permeability-increasing protein in human body fluids by sandwich ELISA. *Journal of Immunological Methods*, **167**, 227–235.

WRIGHT, S. D., RAMOS, R. A., TOBIAS, S., ULETITCH, R. J. and MATHISON, J. C. 1990, CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS-binding protein. *Science*, **249**, 131.