MICROBE EXPOSURE AND THE OCCURRENCE OF ANTIBODIES AGAINST THE EXPOSING MICROBES AMONG WOOD WORKERS IN CELLULOSE INDUSTRY

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ABSTRACT

Exposure to airborne fungal spores and bacteria, and occurrence of antibodies against the most common fungal[14] and bacterial[3] species in sera of the 11 workers were studied in a cellulose factory. Air samples for microbiological analysis were taken by a six-stage Andersen impactor in barking department and on wood chip piles out-of-doors. Barking workers were exposed mainly to bacteria (geometric mean of bacterial concentration 46.3×10^3 cfu/m^3) and to lesser extent to fungal spores (5.9×10^3 cfu/m^3) in contrast to catepillar drivers on wood chip piles (1.5×10^3 cfu/m^3 and 45.5×10^3 cfu/m^3 respectively). Rhodotorula glutinis was the dominating fungal species in the barking department and Aspergillus fumigatus and Penicillium brevicompactum on wood chip piles.

Enzyme-linked immunosorbent assay (ELISA) found differences in IgG-antibody levels between different microbial species as also between different work environments. Highest antibody levels were found against Paecilomyces variotii, Sporobolomyces salmonicolor and Aspergillus niger while lowest levels were found against Rhizopus nigricans, Humicola grisea and Streptomyces albus. Generally, the levels of antibodies against fungal species were 2–5 times higher in the wood chip workers than those in the barking workers. Although the amount of the bacteria in the barking department was about 30 times higher than that on the wood chip piles, no differences in the levels of bacterial antibodies were found between the two groups.

Probably the dry microbial material such as that in wood chip work penetrates into the lower parts of the respiratory tracts and initiates the formation of antibodies more easily than the moist aerosols.

INTRODUCTION

In pulp production wood used as raw material is cutted after barking into chips and stored outdoors in huge piles, wbere chips are transferred by caterpillars. Chips are stored approximately a couple of months before taking in to the production. During the storage microbiological changes occur in the piles (Bergman and Nilsson 1979, Pellikka and Kotimaa 1983). Some cases of allergic alveolitis have been described among wood workers after exposure to fungal spores (van Assendelft et al. 1985, Lundgren and Rosenhall 1979, Jorgensen and Fjellheim 1982). Wood is barked in big barking drums, where water is used in the process. Water is circulated and becomes contaminated by bacteria and fungi. Process is mostly open and water becomes easily aerosolized. Microbial aerosols from humidifiers may cause so-called humidifier fever (Rylander et al. 1978, Marinkovich and Norey 1983). Because of respiratory symptoms among wood workers in a cellulose factory in central Finland, microbe exposure and the antibodies against 17 most common exposing microbes were investigated.

MATERIALS AND METHODS

Subjects and Serum Samples

Serum samples from six catepillar drivers on wood chip piles and four workers in the barking department were taken within two weeks after air sampling. All the examined workers had work-related symptoms suggesting allergic background with microbial etiology (Table I).

Air Sampling for Estimating the Microbe Exposure

Air samples for microbiological analysis were collected by a six-stage fractionating impactor (model 10-800, Andersen Inc., Georgia, USA) (Andersen 1958). Three sets of media were used in successive samplings on each sampling site: Hagen-medium (incubated at 20°C) was used for mesophilic fungi, the same medium incubated at 40°C was used for thermotolerant fungi and plate count agar was used for total count of mesophilic bacteria. 10 air samples for each microbe group were collected both on wood chip piles and in the barking

Table I

Workers' Age, Type of Work, the Duration of Exposure, Symptoms, and the Clinical Findings

ID.CODE	AGE (YRS)	WORK	DUR.OF EXPOSURE (YRS)	SYMPTOMS R E C F M D	CL. FINDINGS PEF	ESR
VA 1	55	CD	25	- - - F M -	NORMAL	12
SA 2	38	CD	8	R - C - - -	NORMAL	2
LA 3	56	CD	14	- - C F M -	NORMAL	12
LI 4	39	CD	14	- - C F - -	NORMAL	5
KU 5	46	CD	26	R - - F - -	LOWERED	17
TO 6	36	WO/S	7/4	R - - - - D	NORMAL	4
KA 7	47	WO/B	4/3	R E - F M -	NORMAL	7
KA 8	37	B	17	R - C - - -	NORMAL	2
MA 9	37	B	15	- - C - - -	NORMAL	2
HA 10	47	B	17	- - C - - -	NORMAL	5

CD = CATERPILLAR DRIVER
WO = WOOD WORKER OUTDOORS
S = SLASHER
B = BARKING WORKER

R = RHINITIS
E = EYE IRRITATION
C = COUGH
F = FEVER
M = MUSCLE PAIN
D = DYSPNEA

ESR = ERYTHROCYTE SEDIMENTATION RATE

department. After incubation the number of colonies was counted, and the positive hole correction method was performed to calculate the concentrations of viable airborne microbes.

Antigens

For antigen preparation 14 fungal end 3 bacterial strains were subcultured from original cultivation plates in nutrient broth containing 5 g/1 peptone (Difco Laboratories, Detroit, Mich., USA) and 3 g/1 beef extract (Difco) at optimal temperature for each species. Bacterial growth was harvested by centrifugation and fungal growth by filtering, and washed three times by distilled water. Microbial pellets were disrupted mechanically (Ultra Turrax, Janke and Kunkel, Staufen i Breisgan, FRG) and then by ultrasonic disintegrator (Soniprep 150, MSE, Crawley, U.K.). The supernatants after a centrifugation at 40,000 g for 30 min were used as ELISA antigens.

Antibody Determination

IgG-antibodies were determinated by enzyme-linked immunosorbent assay (ELISA) carried out on disposable polystyrene microtiter plates (Immumoplate I, Nunc, Denmark). Microbial sonicates were used as antigen and alkaline phosphatase-labelled swine anti-human IgG (Orion Diagnostica, Espoo, Finland) was used as conjugate. Antibody levels were given as ELISA absorbance at a serum dilution of 1:100, read at 405 nm by a Titertek Multiskan (Eflab, Helsinki, Finland).

RESULTS

Microbe Exposure

Marked qualitative differences were found in the microbial exposure of caterpillar drivers and barking workers. Barking workers were exposed mainly to bacteria (geometric mean of bacterial concentration 46.3×10^3 cfu/m^3) and to lesser extent to fungal spores (5.9×10^3 cfu/m^3) in contrast to caterpillar drivers on wood chip piles and 1.5×10^3 cfu/m^3 and 4.5×10^3 cfu/m^3 respectively) (Table II). Rhodotorula glutinis was the dominating fungal species in the barking department, and Aspergillus fumigatus and Penicillium brevicompactum on wood chip piles (Table III).

Antibodies

Differences in antibody levels between different microbial species as also between different work environments were found by ELISA (Table IV). Highest antibody levels were found against Paecilomyces variotii, Sporobolomyces

Table II

Total Concentrations of Airborne Bacteria and Fungi (cfu/m^3) in the Barking Department and on Wood Chip Piles Outdoors ($\bar{x}$ = geometric mean)

	BARKING DEPARTMENT (n=10)		ON WOOD CHIP PILES (n=10)	
MICROBE GROUP	$\bar{x}$	RANGE	$\bar{x}$	RANGE
BACTERIA	46000	9200–230000	1500	770–35000
FUNGI	5900	1400–70000	45000	1000–200000

Table III

Concentrations of Airborne Microbes (cfu/m^3) in the Barking Department and on the Wood Chip Piles Outdoors ($\bar{x}$ = geometric mean)

	BARKING DEPARTMENT (n=10)		ON WOOD CHIP PILES (n=10)	
	$\bar{x}$	RANGE	$\bar{x}$	RANGE
Aspergillus fumigatus	28	0–740	40000	880–200000
Aspergillus niger	2	0–62	9	0–190
Humicola grisea	0	–	9	0–48
Paecilomyces variotii	2	0–41	6	0–110
Penicillium brevicompactum	500	41–3400	6000	12–88000
Rhizopus nigricans	2	0–33	3	0–71
Streptomyces albus	2	0–80	7	0–170
Trichoderma viride	110	17–490	5	0–24
Aureobasidium pullulans	5	0–44	2	0–24
Cephalosporium curtipes	0	–	0	–
Cladosporium cladosporioides	52	0–180	9	0–570
Geotrichum candidum	2	0–21	7	0–150
Phialophora bubakii	0	–	0	–
Rhodotorula glutinis	3900	510–65000	54	0–560
Sporobolomyces salmonicolor	1	0–10	8	0–150
Bacterium 1	930	180–4700	5	0–120
Bacterium 2	33000	8300–210000	9	0–800

Table IV

Antibody Levels ($\bar{x}$ + S.E.) Against the Microbes Found in the Working Environment in Barking Workers and in Wood Chip Workers

MICROBE	BARKING WOS (N=4) $\bar{X}$ + S.E.		WOOD CHIP WOS (N=6) $\bar{X}$ + S.E.	
ASP. FUMIGATUS	0.248	0.057	1.080	0.210
ASP. NIGER	0.477	0.120	1.362	0.242
HUM. GRISEA	0.074	0.011	0.136	0.015
PAEC. VARIOTII	0.408	0.082	1.528	0.103
PENIC. BREVICOMPACTUM	0.157	0.070	1.145	0.201
RHIZ. NIGRICANS	0.115	0.039	0.128	0.033
STR. ALBUS	0.087	0.014	0.172	0.029
TRICH. VIRIDE	0.443	0.107	0.810	0.194
AUR. PULLULANS	0.297	0.074	0.594	0.065
CEPH. CURTIPES	0.683	0.162	1.085	0.094
CLAD. CLADOSPORIOIDES	0.262	0.092	0.853	0.137
GEOTR. CANDIDUM	0.302	0.066	0.469	0.095
PHIL. BUBAKII	0.107	0.029	0.420	0.112
RHODOT. GLUTINIS	0.408	0.040	0.967	0.128
SPOROB. SALMONICOLOR	0.392	0.059	1.377	0.230
BACTERIUM 1	0.425	0.075	0.498	0.066
BACTERIUM 2	0.810	0.254	0.812	0.122

salmonicolor and Aspergillus niger while lowest were found against Rhizopus nigricans, Humicola grisea and Streptomyces albus. Generally, the levels of antibodies against fungal species were 2–5 times higher in the wood chip workers than those in the barking workers. No differences in the levels of bacterial antibodies were found between the two groups.

DISCUSSION

In the cellulose factory, the concentrations of airborne microbes except for bacteria were significantly higher on dusty wood chip piles than in the barking department, and correspondingly, the levels of antibodies against fungal species were higher in the wood chip workers than in the barking workers. Highest antibody levels were found against Paecilomyces variotii, Sporobolomyces salmonicolor, and Aspergillus niger and lowest antibody levels were found against Streptomyces albus, Humicola grisea and Rhizopus nigricans reflecting differences in capability of the species to stimulate a formation of antibodies. Although the amount of the bacteria in the barking department was about 30 times higher because of aerosolized processing water than that on the wood chip piles, no differences in the levels of antibodies against bacteria were found between the caterpillar drivers and the barking workers. Probably the dry microbial material such as that in wood chip penetrates into the lower parts of the respiratory tracts and initiates the formation of antibodies more easily than the moist aerosols.

Fever and muscle pain as work-related symptoms in wood chip workers suggest the diagnosis of allergic alveolitis, which is supported also by high antibody levels in this group (Terho 1982), whereas rhinitis and cough found mostly in barking workers with low antibody levels seem not to be IgG-mediated reactions.

The comparison of antibody findings in the cellulose factory with those in office workers gave a surprising result. The barking workers' antibody levels were not at all higher than those in bank clerks with minimal exposure to airborne microbes (unpubl. data). The dry microbial material occurring in wood chip work and in office work penetrates probably easily into the alveoli and initiates the formation of antibodies more effectively than the moist aerosols irrespective of the amount of antigen.

These results suggest that in addition to microbial concentration the physical nature of aerosols should be considered for evaluating the health risks caused by airborne microbes. On the other hand, the immunization against occupationally exposing microbes could be diminished by controlled air-humidifying to prevent allergic respiratory diseases.

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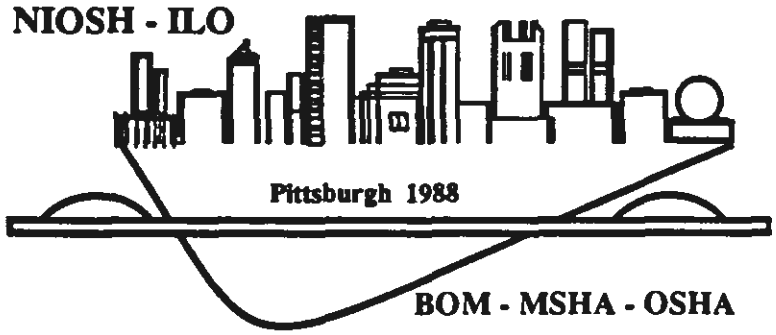

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