Antibody Response to Long-term and High-dose Mould-exposed Sawmill Workers

B. Rydjord*, W. Eduard†, B. Stensby*, P. Sandven‡, T. E. Michaelsen‡,§ & H. G. Wiker¶

*Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo; †Department of Chemical and Biological Working Environment, National Institute of Occupational Health, Oslo; ‡Division of Infectious Disease Control, Norwegian Institute of Public Health, Oslo; §Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Oslo, Oslo; and ¶Department of Microbiology and Immunology, Haukeland University Hospital and The Gade Institute, University of Bergen, Bergen, Norway

Correspondence to: B. Rydjord, Norwegian Institute of Public Health, P.O. Box 4404, Nydalen, N-0403 Oslo, Norway. E-mail: britt.rydjord@fhi.no

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Abstract

Exposure to moulds is thought to cause adverse health effects ranging from vague subjective symptoms to allergy and respiratory diseases. Until now, most studies have been emphasizing low levels of exposure. In Norwegian sawmills during the 1980s, extensively high spore counts up to 10^7 spores/m^3 air were reported. By using serum samples obtained from sawmill workers during that period, in addition to control sera, we studied the antibody response of all classes and IgG subclasses to Rhizopus microsporus at different levels of exposure. Antigen specificity was further studied by Western blotting. Exposure to R. microsporus was accompanied by R. microsporus-specific antibody production against a wide range of antigenic components most likely of both protein and carbohydrate nature. Increasing levels of mould-specific IgG1, IgG2, IgG4 and IgA antibodies were associated with increased exposure, while the highest levels of exposure were associated with a somewhat reduced level of mould-specific IgE antibodies. In conclusion, the present study strongly suggests that high mould exposure can induce a strong IgG and IgA response in a dose-dependent manner.

Introduction

Inhalation of fungal spores has been shown to cause allergic diseases in studies dating back to the late 1970s [1]. Most studies of mould-specific antibody responses have been performed in people residing in water-damaged buildings, exposed to mould spore concentrations ranging from 10 to 10^5 spores/m^3 [2–5]. In sawmills, fungal spore concentrations up to 10^6–10^7 spores/m^3 have been reported [6–8], a much higher concentration than what is normally found in mouldy buildings. In the late 1970s, 27 Swedish sawmills were repeatedly investigated because wood trimmers disease, a type of hypersensitivity pneumonitis, had been diagnosed among Swedish wood trimmers [9], and Aspergillus fumigatus, Rhizopus microsporus and Paezilomyces variotii were found in every sawmill analysed [10]. Rhizopus has been shown to be the most abundant mould in Norwegian sawmills [7, 11], Rhizopus and Penicillium the most abundant in Finnish sawmills [6], while Penicillium has been the predominant mould in Canadian [8] and Swiss sawmills [12].

Heavy mould exposure is found to be a probable cause of respiratory and other work-related symptoms in a dose-dependent manner [7, 13–17], and occupational mould exposure is associated with both respiratory symptoms and elevated levels of mould-specific IgG, IgA and IgM antibodies [16, 18–20]. Antibody subclasses of IgG1, IgG2, IgA1 and IgA2 are all shown to be of importance [18].

During the 1980s, Norwegian sawmill workers were frequently exposed to extensively high spore concentrations, in particular those working in the wood-trimming departments, after introduction of kiln drying and indoor sorting of the timber. Settings where many individuals are exposed to such high spore concentrations of single mould species are rarely observed. Previously, an association has been shown between IgG antibodies to R. microsporus and respiratory symptoms [7, 19] among Norwegian sawmill workers. We therefore wanted to analyse in detail the antibody response of these highly mould-exposed individuals, and study whether any of the antibody (sub)classes to Rhizopus were more strongly
associated with exposure than other antibody (sub)classes. Serum samples from sawmill workers at different departments, in addition to blood donor controls, allowed us to compare the antibody response at different levels of exposure.

Materials and methods

Sera. Twenty-two different sawmills located in Hedmark, an inland county in southern Norway, were investigated during 1987–1988. Blood samples were collected from workers in the various departments in the sawmills, and all participants completed a self-administered questionnaire. In addition, mould spore exposure was measured for wood trimmers in five of the sawmills. Respiratory and other work-related symptoms modelled according to exposure. The lower detection limit of mould spores was about 10^3 spores/m^3 air. The sampling time was one work shift of approximately 8 h. Measurements on several persons at the same days showed very similar values of exposure, making each measurement representative for the exposure that particular day. The timber sorted each day had been dried during different seasons, because the storage time before sorting varied due to logistical reasons. The measurements were therefore performed on several days in each wood-trimming department. Mould exposure in each sawmill was classified as mean Rhizopus exposure.

Mould spore extract. A spore antigen extract was prepared from R. microsporus isolated from a Norwegian sawmill in 1987. The identity of the strain was confirmed at Centraalbureau voor Schimmelcultures, the Netherlands. The mould strain was cultivated on Sabouraud’s dextrose agar for 12 days at 37 °C. Mould material of both mycelium and spores were scraped off the agar, and mixed well with distilled water. Overnight sedimentation allowed the spores to settle, while the mycelium floated and could be removed. The settled material was filtered through a mesh sheet to remove hyphae, and the remaining spores were crushed four times by an X-press (BioTec, Stockholm, Sweden) at −20 °C. Disintegrated spores were centrifuged at 18,000 g, crushed again and centrifuged, before the collected supernatants were freeze dried. Water-soluble spore extract was stored at −70 °C. The protein content was quantified by Bicinchoninic acid assay (BCA™ Protein Assay Kit; Pierce, Rockford, IL, USA) before use.

Table 1 Characteristics of sawmill workers of different departments.

<table>
<thead>
<tr>
<th>Department</th>
<th>n</th>
<th>Age (mean ± SD)</th>
<th>% males</th>
<th>% smokers</th>
<th>% symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sawmill workers</td>
<td>337</td>
<td>45 ± 12</td>
<td>97</td>
<td>45</td>
<td>42</td>
</tr>
<tr>
<td>in total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wood trimmers</td>
<td>99</td>
<td>44 ± 14</td>
<td>95</td>
<td>49</td>
<td>42</td>
</tr>
<tr>
<td>Plane operators</td>
<td>183</td>
<td>45 ± 15</td>
<td>98</td>
<td>40</td>
<td>42</td>
</tr>
<tr>
<td>Other wood workers</td>
<td>42</td>
<td>46 ± 11</td>
<td>100</td>
<td>62</td>
<td>43</td>
</tr>
<tr>
<td>Office workers</td>
<td>13</td>
<td>48 ± 12</td>
<td>100</td>
<td>38</td>
<td>39</td>
</tr>
<tr>
<td>Controls</td>
<td>100</td>
<td>45 ± 13</td>
<td>69</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Other wood workers included saw operators, kiln operators, truck drivers and maintenance personnel. n = number of individuals.
sawmill workers and 100 controls were analysed by an ELISA. MaxiSorp 96-well immunoplates (Nunc, Copenhagen, Denmark) were coated with 0.5 μg of protein spore extract per well, diluted in diethanolaminbuffer with 10% BSA and 0.05% Tween 20, incubated for 1 h at room temperature and stored at 4 °C. Sera and conjugate were diluted in phosphate-buffered saline pH 7.4 (PBS) containing 1% bovine serum albumin (BSA, Biostat AG, Dreieich, Germany), 0.05% Tween 20 and 0.02% NaN₃. Sera were diluted 1:100 for IgE analyses and 1:1000 for IgG1, IgG2, IgG3, IgG4, IgA and IgM. After washing, the samples were incubated with the following secondary antibodies: alkaline phosphatase (ALP)-conjugated monoclonal mouse–anti-human IgG1 (05-3322; Zymed, San Francisco, CA, USA) diluted 1:1000, monoclonal mouse–anti-human IgG2 (05-3522; Zymed) diluted 1:4000, monoclonal mouse–anti-human IgG4 (05-3822; Zymed, San Francisco, CA, USA) diluted 1:4000, polyclonal goat–anti-human IgA (60-110, Fitzgerald Industries International Inc., Concord, MA, USA) diluted 1:16,000, polyclonal goat–anti-human IgM (60-134, Fitzgerald) diluted 1:16,000, biotinylated monoclonal mouse–anti-human IgG3 (locally made 132-C8, specific for the hinge region) diluted 1:6000 and biotinylated monoclonal mouse–anti-human IgE (B603, Nycomed, Oslo, Norway) diluted 1:3000. Both serum and secondary antibodies were incubated for 2 h. After washing, the biotinylated conjugates were amplified with incubation in StreptABC-complex (Dako Cytomation, Glostrup, Denmark) for 1 h. As standard, a coat of ALP (Sigma, St Louis, MO, USA) was diluted fourfold in diethanolaminbuffer with 10% BSA and 0.05% Tween 20 from 1:32,000 in seven dilutions in each plate. Highly purified myeloma proteins of the given antibody (sub)classes were coated on each plate, and used as positive controls of the secondary antibody specificities and the substrate reaction. In advance, the myeloma proteins were purified on DEAE-ion exchange chromatography, NH₄SO₄ precipitation and gel filtration as described previously [22]. The purity was at least 99% judged by agarose gel electrophoresis, immunoelectrophoresis and radial immunodiffusion. The substrate step was performed with 1 mg/ml p-nitrophenyl phosphate disodiumhexahydrat (Sigma) in 10% diethanolaminbuffer, pH 9.8. After 30 min of incubation, OD was measured at 405 nm in a MRX Microplate Reader (Dynatech Laboratories, Chantilly, VA, USA) connected to a PC using BioLinx software (Dynatech Laboratories) for instrument operation and calculation. Between each incubation step, the plates were washed five times using a Dynawasher (Dynatech Laboratories) with PBS, 0.05% Tween 20 and 0.02% NaN₃. All incubations were done at room temperature with gentle shaking, and all samples were applied in duplicates with 100 μl per well. Each value was set as mean of the duplicates. A negative control serum pool (see above) replaced serum samples in two wells on each plate. The background, that was a well consisting of all solutions except serum, was subtracted before calculating the antibody level. Then the antibody levels were calculated on the basis of the ALP-standard curve, and given as an index of the value of the negative control serum pool. No adherence of serum proteins to uncoated wells was observed in the preliminary tests.

SDS-PAGE and Western blotting. Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) was performed with the Pharmacia system for horizontal electrophoresis in a Multifor II electrophoresis unit 2117 (Pharmacia LBK Biotechnology AB, Uppsala, Sweden) with precast polyacrylamid gels (ExcelGel SDS gradient 8–18; Amersham Pharmacia Biotech, Uppsala, Sweden) that were diffusion blotted according to the method by Olsen and Wiker [23]. R. microsporus spore extract was diluted in sample buffer with non-reducing conditions, and applied at 20 μg of protein per lane. The gel was blotted on a NitroBind nitrocellulose transfer membrane (MSI Westboro, MA, USA) for 4 min and stained with Coomassie Brilliant Blue.

The blot was blocked with PBS with 2% BSA, 1% of hipure liquid gelatin (Norland products Inc., New Brunswick, NJ, USA) and 0.02% NaN₃ for 2 h and washed for 30 min with PBS with 0.1% Tween 20 and 0.02% NaN₃. For antibody binding analyses, the blots were incubated with serum diluted 1:500 in PBS with 0.2% BSA and 0.1% gelatin for 2 h in room temperature and overnight at 4 °C. Then the blots were washed, incubated with alkaline phosphate-conjugated anti-human antibodies (the same as in ELISA described above) diluted 1:1000 in PBS with 0.2% BSA and 0.1% gelatin for 2 h, washed and developed with BCIP/NBT alkaline phosphatase substrate (Sigma) and analysed by Kodak Image Station 1000 (Eastman Kodak company, Rochester, NY, USA).

For carbohydrate analyses, blots were blocked, washed and incubated with 2 μg/ml of the following biotinylated lectins; Conconavalin A (ConA, Amersham Pharmacia Biotech), lentil lectin (Sigma), peanut lectin (Sigma) and wheat germ agglutinin (Sigma), diluted in PBS with 0.2% BSA and 0.1% gelatin for 2 h, washed and developed with Western lightning CDP-star chemiluminescence reagent (Perkin-Elmer Life Sciences, Boston, MA, USA) and analysed as described above. ConA, lentil lectin, peanut lectin and wheat germ agglutinin were biotinylated and controlled for specificity as described previously [24].

Statistics. Statistical analyses were performed with SigmaStat Statistical Analysis System for Windows Version 3.1 (Jandel Scientific, Erkrath, Germany) and SPSS Version 13 (SPSS Inc., Chicago, IL, USA). All
antibody data were positively skewed, and therefore log transformed before statistical analysis. Most of the groups were normally distributed after transformation, the others had an approximately symmetrical distribution and similar variances. A one-way ANOVA were used to determine whether the antibody levels differed between groups. If the ANOVA demonstrated significant differences, Tukey’s post hoc test was run to determine which groups differed from the others. Independent sample t-tests were used to compare sawmill workers with the controls, and low versus high mould spore exposure among wood trimmers. Coherence of the antibody levels was analysed by Pearson correlation test on log-transformed data. Univariate linear regression was used for modelling determinants of antibody levels with working group adjusted for gender, age and smoking.

**Results**

Serum samples from the 337 sawmill workers and 100 controls were analysed for *Rhizopus microsporus*-specific antibodies of all classes and IgG subclasses. The different groups showed a positively skewed distribution of all antibodies. Compared with controls, sawmill workers differed significantly in *R. microsporus*-specific antibody levels of all the measured classes and subclasses (Fig. 1). Sawmill workers had significantly higher levels of IgG1 (P < 0.001), IgG2 (P < 0.001), IgG3 (P = 0.02), IgG4 (P < 0.001) and IgA (P = 0.002) than controls, but significantly lower IgM (P = 0.02) and IgE levels (P < 0.001).

The 337 sawmill workers were grouped in working categories due to qualitatively estimated mould exposure; wood trimmers (n = 99), plane operators (n = 183), other wood workers (n = 42) and office workers (n = 13). The overall levels of *Rhizopus*-specific IgG1, IgG2, IgG4 and IgA antibodies were elevated in association with increasing exposure. IgG3 and IgM antibody levels showed only minor differences between differently exposed groups. The level of IgE antibodies were low among all groups, but tended to decrease in association with increasing exposure. Data for IgG1, IgG2 and IgE antibodies are shown in Fig. 2. The levels of different antibody classes and subclasses showed a low-to-moderate degree of correlation within the sawmill workers, with all r ≤ 0.59 and all P < 0.001 (data not shown). Within the sawmill workers, the level of IgG1 correlated positively with IgG2 (r = 0.54), IgG4 (0.55), IgA (0.59) antibodies, and negatively with IgE (r = −0.50). The levels of IgG2 antibodies correlated positively with IgG4 (r = 0.28), IgA (r = 0.42) antibodies, and negatively with IgE antibodies (r = −0.39). The level of IgG4 antibodies correlated positively with IgA antibodies (r = 0.44) and negatively with IgE antibodies (r = −0.23). A negative correlation was seen between IgA and IgE antibodies (r = −0.41). Overall, the trend was similar within the different working groups, with the highest correlations within wood trimmers (r ≤ 0.73).

Mould exposure was measured within five sawmills, but in wood trimmer departments only. A total of 53 mould samples were taken using personal samplers.

![Figure 1](https://via.placeholder.com/150)

**Figure 1** Levels of *Rhizopus microsporus*-specific antibodies of different (sub)classes in controls (white boxes, n = 100) and sawmill workers (grey boxes, n = 337). Antibody levels are given as ratio to a reference serum pool. Box plots show non-transformed median antibody concentrations and 10th, 25th, 75th and 90th percentiles as vertical boxes with error bars. For IgG1 in the control group, the median is identical with the 25th percentile. The statistical analyses with independent sample t-tests were performed on log-transformed data.
The concentration of \( \textit{R. microsporus} \) spores were found to vary greatly between different sawmills. Three sawmills were classified as low exposure sawmills, with mean \( R. \textit{microsporus} \) spore counts of \( 1 \times 10^3, 2 \times 10^3 \) and \( 2 \times 10^4 \) spores/m\(^3\), and a maximum count of \( 6 \times 10^4 \) spores/m\(^3\). Two sawmills were classified as high exposure sawmills, with mean \( R. \textit{microsporus} \) spore counts of \( 2 \times 10^6 \) and \( 3 \times 10^6 \) spores/m\(^3\), and a maximum count of \( 1 \times 10^7 \) spores/m\(^3\). The \( R. \textit{microsporus} \) spore counts corresponded to approximately 40% of the total spore count [7].

Wood trimmers working in the high exposure sawmills had significantly higher levels of \( R. \textit{microsporus} \)-specific IgG1 (\( P = 0.002 \)), IgG2 (\( P = 0.008 \)), IgG3 (\( P = 0.001 \)) and IgA (\( P = 0.005 \)) and significantly lower levels of IgE (\( P = 0.01 \)) antibodies than those working in the low exposure sawmills. Data for IgG1, IgG2 and IgE are shown in Fig. 3. There were no differences in the levels of IgG4 or IgM antibodies between the two groups of wood trimmers (data not shown).

Statistical modelling of \( R. \textit{microsporus} \)-specific antibody level determinants, including sex, age, smoking and type of work, showed that working as a wood trimmer was significantly associated with increased levels of IgG1 and IgG2, and decreased levels of IgE antibodies (Table 2). As we did not have data for smoking in the control group, these tests were performed with comparison to office workers. No significant associations for other groups of workers than wood trimmers were found, but IgG1, IgG2, IgG4, IgA and IgE antibodies tended to associate with exposure levels in a dose-dependent way. Smoking was associated with decreased levels for IgG1, IgG2, IgG4, IgA and IgE antibodies, and age had a small positive effect on IgG2 and IgA levels (data for IgG1, IgG2 and IgE are shown in Table 2). Sex had no significant association with antibody levels in the model. On the other hand, a one-way ANOVA within the control group showed that men had significantly more specific IgG2 (\( P = 0.03 \)), and less specific IgM (\( P = 0.04 \)) than females (data not shown). However, the exclusion of female controls did not influence the significance levels.

Sera showing the highest antibody levels of each (sub)class in the ELISA were selected for further Western blotting studies. These analyses revealed several distinct \( R. \textit{microsporus} \) antigens in the spore extract (Fig. 4). The antibody binding pattern were both individual and (sub)class specific. IgG1 antibodies were directed against several distinct antigens of different molecular weights (Fig. 4, lane b–e). IgG2 antibodies were mainly directed against smears (Fig. 4, lane f–i). IgG3 and IgA antibodies bound to both smears and distinct bands apparently corresponding to the main antigens binding IgG1, while IgM antibodies bound mainly to antigens of small molecular masses (data not shown). IgG4 showed no detectable binding of any antigen (data not shown). ConA reacted to smears in two of the same areas as IgG2 antibodies (Fig. 4, lane j). Lentil lectin, wheat germ agglutinin or peanut lectin did not show significant binding to any \( R. \textit{microsporus} \) components (data not shown).
Discussion

The immune response to moulds has previously been shown to include IgG, IgA and IgE antibody production \[20, 25, 26\]. In the present study, R. microsporus-specific antibodies of all (sub)classes were found, and the highest levels were observed among IgG1 and IgG2 antibodies. The levels of mould-specific antibodies among sawmill workers were related to type of work and thereby to qualitatively estimated mould spore exposure. This was particularly seen for IgG1, IgG2, IgG4, IgA and IgE antibodies. These associations were further supported by the antibody levels among wood trimmers working in high and low exposure sawmills, and by the statistical modelling of determinants influencing the antibody levels.

The level of R. microsporus-specific IgE antibodies was overall low. Still, the levels of R. microsporus-specific IgE correlated negatively with that of R. microsporus-specific IgG and IgA antibodies, and were lower in the wood trimmers than in the less exposed sawmill workers. Correspondingly, the IgE levels were lower in wood trimmers working in sawmills of high mould spore concentrations than those working in low exposure sawmills. A trend of increasing IgG antibody levels at increasing exposure, but with a reduced IgE antibody level among the most exposed individuals, is in accordance with previous observations for other allergens, described as a modified Th2 response \[27–30\]. However, as the blood donor controls had higher levels of specific IgE antibodies than the sawmill workers, we cannot exclude that these observations could be influenced by the inhibition of IgE antibody binding in workers with strong IgG or IgA responses to R. microsporus. It is unknown to what degree blood donors actually are sensitized to a mould like R. microsporus. Moulds exist

Table 2 Multiple linear regression models of determinants related to Rhizopus-specific antibodies in different working groups, given by determined regression coefficients (\(b\)), their standard errors (SE) and \(P\)-values.

<table>
<thead>
<tr>
<th>Determinants</th>
<th>IgG1</th>
<th></th>
<th></th>
<th>IgG2</th>
<th></th>
<th></th>
<th>IgE</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(b)</td>
<td>SE</td>
<td>(P)</td>
<td>(b)</td>
<td>SE</td>
<td>(P)</td>
<td>(b)</td>
<td>SE</td>
<td>(P)</td>
<td></td>
</tr>
<tr>
<td>(r^2_{adj})</td>
<td>0.16</td>
<td>0.22</td>
<td>0.096</td>
<td>0.22</td>
<td>0.005</td>
<td>0.005</td>
<td>0.096</td>
<td>0.005</td>
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<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>2.13</td>
<td>0.26</td>
<td>0.0</td>
<td>1.12</td>
<td>0.39</td>
<td>0.005</td>
<td>2.097</td>
<td>0.023</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>0.17</td>
<td>0.18</td>
<td>0.3</td>
<td>0.50</td>
<td>0.27</td>
<td>0.7</td>
<td>-0.030</td>
<td>0.016</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.003</td>
<td>0.002</td>
<td>0.2</td>
<td>0.011</td>
<td>0.003</td>
<td>0.002</td>
<td>0.0</td>
<td>0.0</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Smoking (yes)</td>
<td>-0.37</td>
<td>0.06</td>
<td>0.0</td>
<td>-0.65</td>
<td>0.10</td>
<td>0.0</td>
<td>0.019</td>
<td>0.006</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wood trimmers</td>
<td>0.33</td>
<td>0.39</td>
<td>0.005</td>
<td>0.97</td>
<td>0.26</td>
<td>0.0</td>
<td>-0.035</td>
<td>0.015</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Plane operators</td>
<td>-0.083</td>
<td>0.16</td>
<td>0.6</td>
<td>0.4</td>
<td>0.25</td>
<td>0.1</td>
<td>-0.009</td>
<td>0.015</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Other wood workers</td>
<td>-0.059</td>
<td>0.18</td>
<td>0.7</td>
<td>0.14</td>
<td>0.08</td>
<td>0.6</td>
<td>0.001</td>
<td>0.016</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Office workers</td>
<td>ref.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The regression coefficients are in bold when \(P < 0.05\). \(r^2_{adj}\) is the variance explained by the models adjusted for the variables used in the model. ref. = reference group.
ubiquitously in nature, but *Rhizopus* species are usually not found in indoor or outdoor air [2, 31]. However, *Rhizopus* species commonly colonize fruit and bread, and both oral and inhalation exposure may occur [32]. Sera from the sawmill workers were obtained in late 1980s, while the blood donor controls were obtained 13 years later. The storage time may have reduced the overall antibody level within sawmill workers, explaining the higher level of specific IgE antibodies among controls. Despite this, the level of IgG and IgA antibodies were still much higher among sawmill workers than the controls, even though these differences thus may have been somewhat underestimated.

The office workers in this study had antibody levels more similar to other sawmill workers rather than to the control group. This indicated that even sawmill office workers were occasionally exposed to mould, and should not without reservation be considered as a non-exposed group. As we did not have any questionnaire data for the blood donor controls, the statistical modelling had to be done with the office workers as a reference. As office workers had antibody levels higher than the blood donor controls, the effect of determinants estimated by statistical modelling may have been underestimated.

The correlation of different antibody (sub)class concentrations among sawmill workers indicated a co-variation that could have been strengthened by cross-reactivity in the assays. However, Western blotting analyses revealed that the antibody (sub)classes were mainly directed to different mould antigens, even though several antigens triggered strong responses of more than one antibody (sub)class. Individual differences were observed for all antibody (sub)classes, with regard to which antigens elicited the highest antibody responses. The Western blotting analyses revealed several distinct antigens, emphasizing that exposure involves complex antigen mixtures. In our Western blotting analysis, both IgG1 and IgA antibodies appeared to be directed to protein antigens as they bound mainly to distinct bands. IgG2 and to some degree IgG3 antibodies binding to smears indicated an immunological reaction to carbohydrate antigens. As the smears also bound ConA, these carbohydrates included mannose and/or glucose residues.

The questionnaire was designed for non-allergic respiratory symptoms, and we were therefore not able to relate the IgE measurements directly to symptoms of allergy. Respiratory and febrile symptoms of the wood trimmers and planing operators included in this study have been evaluated previously [7, 19]. These studies showed that exposures to spores from *R. microsporus*, and likely also from other mould species, were probable causes of respiratory symptoms and chills among sawmill workers in a dose-dependent manner. Our findings indicated that these respiratory health problems among sawmill workers was not mediated by *R. microsporus*-specific IgE molecules, as the most exposed sawmill workers had the lowest levels of specific IgE antibodies.

Spore concentrations in mouldy buildings are usually reported up to $10^3$ spores/m$^3$ air [2–5], while the sawmill workers studied here were exposed to spore concentrations up to $10^7$ spores/m$^3$ air. To our knowledge, no previous studies have reported equivalent antibody data from human exposure to similarly high mould spore concentrations. Since the 1980s, huge improvements to prevent mould exposure among sawmill workers have been done. Only a minimum of workers today are exposed to such high levels of mould spores. Therefore, newer data of such highly exposed populations is hard to obtain.

In summary, this study demonstrated that mould-exposed individuals developed mould-specific antibodies of most classes and subclasses. Exposure to *R. microsporus* involved several distinct antigens and a complex antigen mixture, where the different components mainly induced production of different antibody (sub)classes. The levels of IgG1, IgG2, IgG4, IgA and IgE antibodies were particularly related to the type of work and thereby to the level of mould spore exposure. Increased *R. microsporus*
exposure was associated with higher levels of specific IgG and IgA antibodies in a dose-dependent manner, while
the highest levels of exposure were associated with a somewhat reduced measurements of mould-specific IgE
antibodies.

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