Exposure to Microorganisms Associated With Allergic Alveolitis and Febrile Reactions to Mold Dust in Farmers*

Per Malmberg, M.D.; Anna Rask-Andersen, M.D.; and Leif Rosenhall, M.D., F.C.C.P.

**Study objective:** To compare exposure to microorganisms associated with allergic alveolitis (AA) and with febrile reactions to inhaled mold dust (organic dust toxic syndrome [ODTS]) in farmers and in normal subjects.

**Design:** A prospective study in which exposure was evaluated within two weeks of medical consultation for AA or ODTS. Samples were collected during normal farming (background) and during the handling of materials associated with disease or causing maximal exposure in reference farms (worst case).

**Setting:** Swedish farms

**Participants:** Eleven farmers with a confirmed diagnosis of AA from ten farms, 16 subjects with symptoms of ODTS from 12 farms, and 17 reference farmers.

**Measurements and results:** Worst-case samples representative of the exposure preceding disease were obtained on four farms where five farmers had had AA; the samples contained on average 2.6±1.8×10³ (SD) spores/m³ of air. On six farms where nine farmers had had ODTS, representative samples averaged 13±13×10³ spores/m³, and on reference farms this figure was 0.18±0.50×10³ spores/m³.

The daily spore dose associated with allergic alveolitis was 2×10³ spores/d, which was ten times higher than on reference farms. The average dose associated with ODTS was 2×10⁸ spores. Worst-case samples, collected during 10 to 30 min, contributed to more than 90 percent of the day exposure on farms where AA or ODTS had occurred.

**Conclusion:** Allergic alveolitis was associated with high exposure levels on most weekdays for weeks, and ODTS was associated with extreme exposure occurring on a single day. There was no correlation with individual spore types and disease and the present results are compatible with a hypothesis that common cell wall components of microorganisms may cause "toxic" symptoms and stimulate immune reactions.

(AAA) AA = allergic alveolitis; cfu = colony-forming units; ODTS = organic dust toxic syndrome

The yearly incidence of allergic alveolitis (AA), defined according to strict criteria, is low among Scandinavian farmers (about 2 to 4 cases per 10,000 farmers per year). However, 30 to 50 times more farmers experience febrile reactions to the inhalation of mold dust (organic dust toxic syndrome [ODTS]) than experience AA. Febrile reactions may occur in subjects without previous exposure to mold dust following inhalation of large quantities of microorganisms or other components of organic dusts.

The condition has been given several names, such as ODTS, inhalation fever, and toxic alveolitis. The purpose of the present investigation was to study exposure to microorganisms associated with AA and to compare this exposure to those types encountered in normal farming and in farm dust reported to have caused febrile reactions that do not meet the criteria of AA. The study was designed as a prospective investigation. The farms of farmers with suspected AA or with febrile symptoms were visited within two weeks of the time of notification, and exposure to microorganisms in the time period preceding the disease was evaluated. If possible, the exposure was reproduced and measured.

The results of the present study suggest that inhalation of very high concentrations of mold spores may cause febrile reactions also in nonsensitized subjects. Repeated high exposures to mold dust were associated with AA. The adjuvant property of inhaled spores may be an important determinant of the subsequent immunization leading to AA.

**Materials AND Methods**

**Allergic Alveolitis and Febrile Reactions**

The investigation was designed as a prospective study which lasted for 28 months. Physicians in all departments of internal medicine, lung medicine, occupational medicine, and infectious diseases; district medical centers; and health care centers in Sweden were requested to participate in this study. The physicians were asked to report to the project all new cases of probable AA and cases involving farmers with febrile reactions to mold dust who did not meet the criteria of AA (febrile reaction group). A reminder was sent one year later to the physicians. When a case occurred, it was reported to the project team, and the patient's workplace immediately was contacted in order to secure information regarding exposure. The workplace was then visited within one or two weeks by a microbiologist who measured the exposure and by a physician mentioned.

---

*From the Respiratory Division, National Institute of Occupational Health, Stockholm, Sweden (Dr. Malmberg); the Department of Occupational Medicine and Department of Clinical Physiology, University Hospital, Uppsala, Sweden (Dr. Rask-Andersen); and the Department of Lung Medicine, University Hospital, Umeå, Sweden (Dr. Rosenhall).

Supported by grants 83-0153, 84-0692, 85-1236, 85-0506 and 86-1226 from the Swedish Work Environment Fund.

Manuscript received May 22; revision accepted August 25.

(P.M. or A.R.-A.) acquainted with farming methods and farming health problems who evaluated the symptoms and their relationship to exposure. The aim was to visit all patients with clinically verified AA and those patients in the febrile reaction group for whom the exposure could be measured.

**Reference Farmers**

In a separate study, farmers from dairy farms were randomly selected from the vicinity of Stockholm. All denied a history of AA, and none had experienced febrile reactions to dust during the previous year and were thus considered eligible as references. Exposure to microorganisms toward the end of the indoor season was measured on 17 farms.

**Exposure Measurement**

The first exposure sample was measured during work activities normal for dairy farms, i.e., during feeding, milking, and cleaning (background sample). A second sample was taken during the handling of the material which was believed to have caused symptoms or disease (worst-case sample). If possible, the material was handled in the same way as when the farmer became ill. On reference farms and on those farms where the relationship between symptoms and exposure was uncertain, the worst-case exposure was measured while using the material which was believed to cause the highest exposure to microorganisms. When worst-case samples from symptom-causing farms were obtained, a note was made as to whether or not the exposure could be considered representative of the exposure during the time period in which the disease evolved. Background samples were collected within 1 to 2 h and worst-case examples within 5 to 15 min.

The samples were collected by means of personal samplers. The farmer or the investigator carried three filter cassettes directed obliquely downward on the chest. The filter cassettes were equipped with polycarbonate filters with a pore size of 0.4 µm (Nuclepore Corp., Pleasanton, Cal). The airflow was 1 L/min, and the filter cassettes were used closed-phase. Fluorescence microscopy was used for enumeration of the total number of spores from molds, actinomycetes, and bacteria. In addition, the number of colony-forming units (cfu) was counted and viable microorganisms were characterized as described earlier at the Department of Microbiology, University of Agriculture, Uppsala, Sweden, and at the National Institute of Occupational Health, Umeå, Sweden. The results from the two parallel samples from each side of the chest were averaged. The second filter on the left side was analyzed for the total number of spores of molds or actinomycetes by use of an electron microscope at the National Institute of Occupational Health, Solna, Sweden.

**Laboratory Methods**

For measuring antibodies against molds and actinomycetes, the double diffusion test of Ouchterlony was used. The analyses were performed in the Laboratory of Occupational Allergy at Sahlgrenska Hospital in Gothenburg, Sweden. The panel consisted of the following antigens: *Microsporopsis faeni*, *Thermoactinomyces vulgaris*, *Aspergillus fumigatus*, Alternaria species, Botrytis species, Cladosporium species, Mucor species, Paecilomyces species, Penicillium species, Rhizopus species, and Pullularia species.

 Enumeration and classification of microorganisms were performed within 1 to 2 days after sampling. The spores were extracted from the filters with use of 0.1 percent wt/vol peptone water with 0.01 percent Tween 80. One part of the extract was used for determination of viable microorganisms by the plate count method. Malt-agar plates with penicillin and streptomycin to inhibit bacterial growth were used as a fungal medium. Bacteria and actinomycetes were grown on nutrient agar with Actidione to prevent fungal growth. The number of colony-forming units was recorded after four days on plates cultured at an elevated temperature and after seven days on plates cultured at room temperature (21 to 24°C). Molds were grown at room temperature and 45°C and actinomycetes, at room temperature and 55°C. The highest counts of molds and the highest counts of actinomycetes bacteria were added to give the total number of colony-forming units.

Another part of the extract was used for enumeration of the total number of spores. The spores in the aqueous extracts were fixed with formalin, stained with acridine orange, and filtered through a polycarbonate filter dyed with Sudan black. The total number of spores (single spores and spores in aggregates) was determined by use of a fluorescence microscope.

The filters were prepared for electron microscopy by gold-plating in a JEOL FC1100 sputter. The total number of spores (single spores and spores in aggregates) was counted by use of a JEOL JSM 840A sweep electron microscope. The most common types of spores were classified, and the proportions of actinomycetes and mold spores were counted on the basis of morphologic criteria.

**Criteria for the Diagnosis of Allergic Alveolitis and Febrile Reactions**

The medical records of all subjects reported to the study as

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Smoking</th>
<th>Lung Function</th>
<th>Precipitating Antibodies</th>
<th>Material</th>
<th>Spores/m³</th>
<th>True Exposure % Actinomycetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA 1</td>
<td>1</td>
<td>M</td>
<td>51</td>
<td>Decreased</td>
<td>P</td>
<td>Straw</td>
<td>1.9 x 10³</td>
</tr>
<tr>
<td>AA 2</td>
<td>1</td>
<td>F</td>
<td>45</td>
<td>Normal</td>
<td>+</td>
<td>Straw</td>
<td>1.9 x 10³</td>
</tr>
<tr>
<td>AA 3</td>
<td>3</td>
<td>M</td>
<td>38</td>
<td>Decreased</td>
<td>P</td>
<td>Wood chips</td>
<td>3.6 x 10³</td>
</tr>
<tr>
<td>AA 4</td>
<td>4</td>
<td>M</td>
<td>45</td>
<td>Restrictive</td>
<td>+</td>
<td>Hay</td>
<td>4.5 x 10³</td>
</tr>
<tr>
<td>AA 5</td>
<td>5</td>
<td>M</td>
<td>53</td>
<td>Decreased</td>
<td>+</td>
<td>Straw</td>
<td>2.2 x 10³</td>
</tr>
<tr>
<td>AA 6</td>
<td>6</td>
<td>M</td>
<td>62</td>
<td>Decreased</td>
<td>+</td>
<td>Straw</td>
<td>4.6 x 10³</td>
</tr>
<tr>
<td>AA 7</td>
<td>7</td>
<td>M</td>
<td>60</td>
<td>Decreased</td>
<td>+</td>
<td>Straw</td>
<td>5.9 x 10³</td>
</tr>
<tr>
<td>AA 8</td>
<td>8</td>
<td>M</td>
<td>43</td>
<td>Decreased</td>
<td>+</td>
<td>Straw</td>
<td>3.2 x 10³</td>
</tr>
<tr>
<td>AA 9</td>
<td>9</td>
<td>M</td>
<td>48</td>
<td>Decreased</td>
<td>+</td>
<td>Straw</td>
<td>8.2 x 10³</td>
</tr>
<tr>
<td>AA 10</td>
<td>10</td>
<td>M</td>
<td>54</td>
<td>Decreased</td>
<td>+</td>
<td>Straw</td>
<td>6.9 x 10³</td>
</tr>
<tr>
<td>AA 11</td>
<td>11</td>
<td>F</td>
<td>53</td>
<td>Decreased</td>
<td>+</td>
<td>Straw</td>
<td>4.0 x 10³</td>
</tr>
</tbody>
</table>

*NS = never smoker; XS = ex-smoker (more than 6 months); S = smoker (>1 cigarette/d).
†Ellipses indicate missing information.
‡Restrictive if total lung capacity <85% of predicted or if VC <90% of predicted and FEV₁/VC ratio is normal; obstructive if FEV₁/VC ratio is <80% predicted; otherwise, normal lung function.
§(+ ) = trace amounts of precipitating antibodies or positive test in extended panel.
having suspected cases of AA and febrile reactions caused by mold dust were analyzed. At admission to the hospital the lung function was considered restricted if the total lung capacity was below 85 percent, or vital capacity (VC) was below 80 percent in combination with a normal FEV1/VC ratio, compared with local reference values, or if the diffusing capacity for carbon monoxide (Dco) was below the local normal range in subjects without obstructive airway changes. The criteria for obstructive changes was a FEV1/VC ratio less than 80 percent of predicted.

The diagnosis of AA was considered confirmed if: (1) there was no evidence of other diseases which could have caused the symptoms; (2) there were radiographic changes, symptoms (chills or fever reactions and/or cough and dyspnea) and evidence of restrictive impairment of lung function as defined previously; and (3) there was serologic evidence of immune stimulation by microorganisms or bronchoalveolar lavage findings compatible with the diagnosis.

Febrile reactions in subjects with no evidence of AA were diagnosed in persons who were subjectively healthy prior to the incidence, had a quick and complete recovery (within 1 week, usually within a few days), and typical symptoms occurring 4 to 8 h after exposure to large quantities of organic dust. The main symptoms are moderate or high fever and chills, often combined with breathing difficulties, muscle aches, and cough. Only patients whose symptoms were severe and clearly related to exposure were considered. Slight transient changes in chest radiographs taken shortly after heavy exposure to mold dust were considered compatible with the diagnosis.

### Statistical Methods

Statistical significance was tested by the Mann-Whitney U test. In addition, linear regression was performed with the use of the Statview program.

### Allergic Alveolitis

Eighteen patients were reported with a preliminary diagnosis of AA caused by mold dust. In two farms, the workplaces had been completely cleaned before notification to the project, and these farmers were not further evaluated. Based on evaluation of the clinical data, the diagnosis was confirmed in 11 of the remaining 16 farmers (Table 1). Two farmers with AA who became ill at approximately the same time were from the same farm. In five cases, the diagnosis of AA was not confirmed. In two of these cases, the diagnosis was considered unlikely; in one, the medical investigation was insufficient for diagnosis, and in two cases AA probably was present. However, all criteria for the diagnosis were not met (Table 2).

### Febrile Reactions

Thirty-six subjects were reported as having possible

---

**Table 2—Clinical and Exposure Data in Cases in Which the Diagnosis of Allergic Alveolitis Was Not Confirmed**

<table>
<thead>
<tr>
<th>Workplace</th>
<th>Patient No.</th>
<th>Sex</th>
<th>Age, yr</th>
<th>Smoking Habit</th>
<th>Dco</th>
<th>PaO₂, mm Hg</th>
<th>Lung Function</th>
<th>Precipitating Antibodies</th>
<th>Material</th>
<th>Spores/m²</th>
<th>True Exposure</th>
<th>% Actinomyces</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA 2</td>
<td>12</td>
<td>M</td>
<td>25</td>
<td>NS</td>
<td>Decreased</td>
<td>7.3</td>
<td>Obstructive</td>
<td>-</td>
<td>Hay</td>
<td>3.6·10⁶^a</td>
<td>Higher</td>
<td>&lt;1</td>
</tr>
<tr>
<td>AA 3</td>
<td>13</td>
<td>M</td>
<td>46</td>
<td>NS</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>Normal</td>
<td>?</td>
<td>?</td>
<td>1.3·10⁶</td>
<td>Similar</td>
<td>&lt;1</td>
</tr>
<tr>
<td>AA 6</td>
<td>14</td>
<td>M</td>
<td>70</td>
<td>NS</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>(? )</td>
<td>?</td>
<td>7.8·10⁶</td>
<td>?</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>AA 8</td>
<td>15</td>
<td>M</td>
<td>42</td>
<td>NS</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>(? )</td>
<td>Hay</td>
<td>1.7·10⁶</td>
<td>Similar</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>AA 10</td>
<td>16</td>
<td>M</td>
<td>57</td>
<td>XS</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>Restrictive</td>
<td>-</td>
<td>Straw</td>
<td>2.3·10⁶</td>
<td>?</td>
<td>87</td>
</tr>
</tbody>
</table>

For abbreviations, see Table 1.

---

**Table 3—Clinical and Exposure Data of the Febrile Reaction Group**

<table>
<thead>
<tr>
<th>Workplace</th>
<th>Patient No.</th>
<th>Sex</th>
<th>Age, yr</th>
<th>Smoking Habit</th>
<th>Dco</th>
<th>PaO₂, mm Hg</th>
<th>Lung Function</th>
<th>Precipitating Antibodies</th>
<th>Material</th>
<th>Spores/m²</th>
<th>True Exposure</th>
<th>% Actinomyces</th>
</tr>
</thead>
<tbody>
<tr>
<td>FR 1</td>
<td>17</td>
<td>M</td>
<td>29</td>
<td>S</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>. . .</td>
<td>-</td>
<td>Oats</td>
<td>3.3·10⁶</td>
<td>Similar</td>
<td>. . .</td>
</tr>
<tr>
<td>FR 2</td>
<td>18</td>
<td>M</td>
<td>28</td>
<td>XS</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>. . .</td>
<td>-</td>
<td>Oats</td>
<td>3.3·10⁶</td>
<td>Similar</td>
<td>. . .</td>
</tr>
<tr>
<td>FR 3</td>
<td>19</td>
<td>F</td>
<td>25</td>
<td>XS</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>. . .</td>
<td>-</td>
<td>Oats</td>
<td>3.3·10⁶</td>
<td>Similar</td>
<td>. . .</td>
</tr>
<tr>
<td>FR 4</td>
<td>20</td>
<td>M</td>
<td>54</td>
<td>NS</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>. . .</td>
<td>Barley/Oats</td>
<td>6.2·10⁶</td>
<td>Higher</td>
<td>. . .</td>
<td>69</td>
</tr>
<tr>
<td>FR 5</td>
<td>21</td>
<td>M</td>
<td>48</td>
<td>NS</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>. . .</td>
<td>Wood chips</td>
<td>5.1·10⁶</td>
<td>Similar</td>
<td>&lt;1</td>
<td>. . .</td>
</tr>
<tr>
<td>FR 6</td>
<td>22</td>
<td>M</td>
<td>32</td>
<td>NS</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>. . .</td>
<td>Barley</td>
<td>5.9·10⁶</td>
<td>Higher</td>
<td>&lt;1</td>
<td>. . .</td>
</tr>
<tr>
<td>FR 7</td>
<td>23</td>
<td>M</td>
<td>65</td>
<td>S</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>. . .</td>
<td>Barley</td>
<td>5.8·10⁶</td>
<td>Higher</td>
<td>&lt;1</td>
<td>. . .</td>
</tr>
<tr>
<td>FR 8</td>
<td>24</td>
<td>M</td>
<td>60</td>
<td>Normal</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>. . .</td>
<td>Barley/feats</td>
<td>1.7·10⁶</td>
<td>Similar</td>
<td>38</td>
<td>. . .</td>
</tr>
<tr>
<td>FR 9</td>
<td>25</td>
<td>M</td>
<td>42</td>
<td>XS</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>. . .</td>
<td>Oats</td>
<td>1.5·10⁶</td>
<td>?</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>FR 10</td>
<td>26</td>
<td>M</td>
<td>52</td>
<td>Decreased</td>
<td>10.2</td>
<td>Normal</td>
<td>(? )</td>
<td>Oats</td>
<td>Silage</td>
<td>5.0·10⁶</td>
<td>Higher</td>
<td>85</td>
</tr>
<tr>
<td>FR 11</td>
<td>27</td>
<td>M</td>
<td>37</td>
<td>NS</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>. . .</td>
<td>Barley</td>
<td>3.6·10⁶</td>
<td>Similar</td>
<td>59</td>
<td>. . .</td>
</tr>
<tr>
<td>FR 12</td>
<td>28</td>
<td>M</td>
<td>27</td>
<td>NS</td>
<td>8.8</td>
<td>. . .</td>
<td>Barley/peas</td>
<td>1.6·10⁶</td>
<td>Similar</td>
<td>96</td>
<td>. . .</td>
<td></td>
</tr>
<tr>
<td>FR 13</td>
<td>29</td>
<td>M</td>
<td>27</td>
<td>NS</td>
<td>9.8</td>
<td>. . .</td>
<td>Barley/peas</td>
<td>1.6·10⁶</td>
<td>Similar</td>
<td>96</td>
<td>. . .</td>
<td></td>
</tr>
<tr>
<td>FR 14</td>
<td>30</td>
<td>M</td>
<td>50</td>
<td>Decreased</td>
<td>9.1</td>
<td>Obstructive</td>
<td>(? )</td>
<td>Oats</td>
<td>Straw</td>
<td>3.2·10⁶</td>
<td>?</td>
<td>&lt;1</td>
</tr>
<tr>
<td>FR 15</td>
<td>31</td>
<td>M</td>
<td>49</td>
<td>NS</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>. . .</td>
<td>Oats</td>
<td>3.7·10⁶</td>
<td>Similar</td>
<td>91</td>
<td>. . .</td>
</tr>
<tr>
<td>FR 16</td>
<td>32</td>
<td>M</td>
<td>33</td>
<td>S</td>
<td>. . . . . .</td>
<td>. . .</td>
<td>. . .</td>
<td>Barley</td>
<td>2.1·10⁶</td>
<td>?</td>
<td>. . .</td>
<td>. . .</td>
</tr>
</tbody>
</table>

*When two materials were mixed together, both are given. For abbreviations, see Table 1.

---

*1204 Exposure to Microorganisms Associated with Allergic Alveolitis (Malmberg, Rask-Andersen, Rosenhall)
to 3. All farmers with AA had a history of progressive dyspnea and malaise for periods of weeks or months prior to seeking medical advice. The PaO₂ measured in the acute stage of the disease was lower in patients with AA (n = 8) than in the febrile reaction group (n = 4), namely 8.0 ± 1.1 (SD) versus 9.5 ± 0.64 (SD) mm Hg (p < 0.01). One diagnostic criterion for the febrile reaction group was absence of symptoms of lung disease prior to the medical consultation. Similarly, other differences between the groups (chest radiography, precipitating antibodies, restrictive lung function) were influenced by the selection of cases to the groups. In one case, precipitating antibodies were not demonstrated against the antigens in the standard panel, but history and clinical findings, including bronchoalveolar lavage, were typical of AA. Chest radiographs were taken within two weeks of exposure in eight subjects from the febrile reaction group. Three had minimal shadows which had disappeared at follow-up.

### Spore Levels

Individual spore concentrations (worst cases, fluorescence microscopy) are given in Tables 1 to 3 and in Figure 1. Measured exposure was considered to be representative of the exposure that prevailed in the period preceding the disease in four workplaces where five subjects had acquired AA (Table 1), and in six workplaces where nine subjects had experienced febrile reactions (Table 2).

The mean spore concentrations in the different groups are shown in Table 4. The spore concentration in representative worst-case samples was about 20 times higher in the AA group than on reference farms (p < 0.001) and 110 times higher in the febrile reaction group than on reference farms (p < 0.001). The spore level in the febrile reaction group was significantly higher than in the AA group (representative samples, p < 0.05).

### Background Samples

There were no significant differences in mean spore concentrations in air collected from the respiratory zone during farm work and total spore dose during one day.

### Clinical Findings

The main clinical findings are presented in Tables 1 to 4.

**Figure 1**. Spore concentration in air samples taken during the handling of farm material (worst-case samples). Open circle, sample from symptomless farmer. Solid circle, sample representative of the exposure associated with disease. Triangle, sample probably underestimating the exposure preceding disease. Square, sample of uncertain representativity. Samples from five farms where possible cases of AA occurred, but where the diagnosis was not confirmed, are shown separately. A plus sign or question mark indicates probable or questionable diagnosis within this group. Cases of febrile reactions to mold dust. At five workplaces, more than one person was exposed and all exposed persons became ill. The first 16 patients with febrile reactions reported to the project were visited, and the exposure was measured. The results of the medical investigation and the exposure measurements from these cases are shown in Table 3.

### Table 4—Spore Concentration in Air Collected From the Respiratory Zone During Farm Work and Total Spore Dose During One Day *

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Farm, Sample</th>
<th>Worst Case -10⁶ spores/m³†</th>
<th>Background -10⁶ spores/m³</th>
<th>Total Day Dose -10⁶ spores/d‡</th>
<th>Worst case dose Total, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Febrile reactions</td>
<td>All farms</td>
<td>7.0 (10.9) 12</td>
<td>0.009 (0.015) 4</td>
<td>23.3 (6.0-75.6)</td>
<td>99.8</td>
</tr>
<tr>
<td></td>
<td>Representative samples</td>
<td>13.5 (12.6) 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>All farms</td>
<td>1.4 (1.6) 10</td>
<td>0.021 (0.013) 6</td>
<td>2.0 (0.5-3.3)</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Representative samples</td>
<td>2.6 (1.5) 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference farms</td>
<td>All farms</td>
<td>0.1 (0.6) 17</td>
<td>0.015 (0.017) 8</td>
<td>0.2 (0.04-0.8)</td>
<td>48</td>
</tr>
</tbody>
</table>

*Worst case samples were collected during the handling of material probably associated with disease or believed to cause the highest exposure to microorganisms (reference farmers). Background samples were taken during other normal dairy farm operations. Representative samples denotes samples believed to be representative of the exposure preceding disease.

†Mean (SD) number of farms.
‡Mean (range).
concentrations in background samples among the three groups. The spore concentrations in background samples were much lower than in worst-case samples.

**Estimated Total Exposure**

Based on the case histories, the average duration of exposure in worst-case situations was estimated to be approximately 60 min in the febrile reaction group (range, 5 to 180 min). This exposure occurred on one day or at most on two consecutive days. Exposure was caused by unusual work tasks and was not representative of day-to-day exposure. The most typical operation was removal of grain which had been inadequately conserved and undergone spontaneous heating, resulting in excessive growth of microorganisms.

Farmers who had acquired AA had been exposed to the worst-case type of dust for an average of 15 to 30 min on one or two occasions per day. As a rule, this type of exposure had taken place every day for several weeks prior to the illness. In most cases, the offending dust was generated when the farmer handled materials which were very moldy due to spontaneous heating. Typical operations associated with AA were distributing moldy bedding material (straw), handling moldy wood chips (used as fuel), or feeding cows with moldy hay.

The daily spore exposure was estimated for each group by assuming an average ventilation of 35 L/min (corresponding to a moderate to heavy work load). This was multiplied by the individual estimates of duration of exposure and spore concentrations in worst-case samples. The spore exposure during “background” barn work was similarly calculated, assuming 3 h of background exposure each day.

The results of the calculations are shown in Table 4. The worst case samples represented about half of the daily exposure on the reference farms and 90 percent on farms where AA had occurred and 99.8 percent on the day of symptoms on febrile reaction farms. The estimated day exposure in the AA group was approximately 10 times higher and more than 100 times higher in the febrile reaction group than on reference farms.

**Comparison Between Different Methods of Measuring Spore Concentration: Presence of Spore Aggregates**

There was no significant difference in the spore concentration as measured by the electron microscope and by fluorescence microscopy. The proportion of actinomycetes spores was determined by electron microscopy. Total spore counts were taken from the fluorescence microscopy counts. Substituting total spore counts with data from electron microscopy counts did not influence the conclusions.

The number of colony-forming units was consider-ably smaller than the total number of spores. Thus, the concentration of colony-forming units in background and worst-case samples corresponded to 8 and 27 percent, respectively of the total number of spores as measured by the fluorescence or electron microscopic methods. Based on electron microscopy, we found that an average of 89 percent of spores or spores in aggregates were less than 10 μm in physical diameter and thus probably of respirable size.

**Spore Types**

The most common mold spore types in representative samples from farms where patients with AA and febrile reactions worked were Aspergillus, Penicillium, Rhizomucor, and Wallemia. *Aspergillus fumi*ga*tus* was found in six out of ten representative samples but was not predominant in any sample. Spores of *Aspergillus umbrosus* were found in three samples from farms where patients had febrile reactions and in no samples from farms where patients had AA, according to the results of culture in salt-malt agar or using morphologic criteria in electron microscopy. In five representative samples from farms where those in the AA group worked, no actinomycetes were found, but in other samples the majority of spores were composed of actinomycetes. The actinomycetes were primarily of the Streptomyces type.

**Discussion**

The concentrations of mold spores or actinomycetes, or both, were very high in representative samples from farms of the two disease groups compared with levels observed in normal farming. The most conspicuous difference between the disease groups was that exposure associated with AA had occurred on most days for weeks, while the febrile reactions (ODTS) were observed following a single occurrence of exceptional exposure. One out of 11 farmers with a confirmed diagnosis of AA had, however, an exposure history and measurements which appeared normal for dairy farming. More than 90 percent of the exposure occurred during a relatively short period of time (5 to 15 min) when the farmer was handling material which had undergone spontaneous heating, causing excessive growth of microorganisms.

Farmers with AA had symptoms which progressed gradually over weeks prior to admission. Recovery was slow and often incomplete. In seven cases, multiple bronchoalveolar lavage was performed, demonstrating lymphocytosis, very high mast cell counts, and high levels of hyaluronic acid in the bronchoalveolar lavage fluid.12-14

In five cases, the preliminary diagnosis of AA was not confirmed, and in three of these cases the diagnosis of AA was questionable. The latter three cases had relatively low exposure to mold dust.
The daily spore dose associated with a confirmed diagnosis of AA was on average about ten times higher than on reference farms. The asymptomatic reference farmers were, however, also exposed to high spore levels, although less than in the symptomatic groups. Ten were studied with bronchoalveolar lavage. The results indicated the presence of asymptomatic alveolitis with high lymphocyte counts, increased production of inflammatory mediators in lavage fluid, and increased titer of antibodies against mold antigens in serum. These findings are consistent with those of earlier studies.

The finding that AA in farmers is associated with repeated exposure to large numbers of spores is not surprising. Many reports comment on the association between allergic alveolitis and the handling of moldy material or factors promoting moldiness, such as rainfall. Higher levels in AA farms compared with reference farms have been reported from Finland, although the levels were much lower than in the present study, mostly due to differences in sampling strategy and differences between counts expressed in colony-forming units and total spore counts.

It is not possible to exclude the possibility that some of the subjects in the febrile reaction group had subclinical AA, but several observations indicate that these febrile reactions also occurred in subjects who were not sensitized to mold dust. All subjects in the febrile reaction group had a history of normal health prior to the attack, and their condition returned to normal within a short time after the attack. In the five farms where more than one person participated in the work, all had symptoms, including one visiting non-farmer. This clustering of cases also has been observed in other studies. The exposure was due to unusual activities (such as removing moldy material), and on other workdays the exposure appeared normal for farming. Although the acute symptoms can be quite severe, the functional changes were not as pronounced as in patients with AA. An earlier study showed that farmers who have experienced febrile reactions of the ODTS type have normal gas exchange at rest and during exercise and normal lung function compared with reference farmers following recovery.

The febrile reactions were preceded by very high exposure to mold or actinomycetes spores, or both. The spore composition was highly variable on farms where AA or febrile reactions had occurred, and it was not possible to point to any particular mold or actinomycetes as the main cause of either of the two diseases. Assuming that there are $0.3 \times 10^6$ alveoli in human lungs and about 5 to $8 \times 10^9$ alveolar macrophages in the lungs of nonsmokers, the total exposure associated with febrile reactions corresponds to about 80 times more spores than there are alveoli or about 4 times more spores than the number of alveolar macrophages. Spores of the Aspergillus type are able to partly resist phagocytosis by macrophages. Spores have been cultured from a human biopsy sample one day after exposure and from bronchoalveolar lavage fluid many days after exposure. Thus, exposures associated with febrile reactions represent a considerable challenge to the defense system of the lungs.

Most of the farmers with AA had experienced febrile reactions. Thus, a lower dose may have provoked a febrile reaction in farmers with AA than in nonsensitized farmers. However, the difference in the dose of mold dust causing a similar degree of fever appears to be on the order of a factor of ten or less. Thus, febrile symptoms in AA possibly could have been caused by mechanisms similar to those that caused fever in apparently nonsensitized subjects. Bronchoalveolar lavage indicates that both in AA and in apparently healthy farmers, acute exposure to microorganisms may cause a reaction characterized by neutrophil invasion.

It has been suggested that febrile reactions in nonsensitized subjects following inhalation of organic dusts (ODTS) could result from release of interleukins from activated alveolar macrophages. Complement activation by the direct or indirect way may attract and activate inflammatory cells. The cell walls of most mold and yeast spores contain β-(1-3)-D-glucan, which in vitro activates alveolar macrophages following interaction with a specific receptor, causing the release of interleukin-1 and tumor necrosis factor. Endotoxin is a constituent of the outer cell wall of Gram-negative bacteria. Inhalation of endotoxin causes fever, probably due to activation of the endotoxin-receptor on alveolar macrophages. Molding is, however, characterized by an enormous increase in mold spores or actinomycetes which do not have endotoxin. The endotoxin concentration was measured in the present study (data not shown), but this resulted in aberrant values, probably as a result of β-(1-3)-D-glucan interfering with the endotoxin assay. Actinomycetes are Gram-positive bacteria with a cell wall backbone made up of peptidoglycans. The peptidoglycan receptor on alveolar macrophages is, however, much less studied than the glucan and endotoxin receptors, and it is not known if peptidoglycans may contribute to febrile reactions. A possible role of mycotoxins also has been discussed.

Peptidoglycan fragments and β-(1-3)-D-glucan stimulate immune reactions and can be used instead of mycobacteria in Freund's adjuvant. Also endotoxin has adjuvant effects. Intratracheal instillation of $10^6$ spores of A fumigatus on one occasion caused granulomatous changes in the lungs of rats 3 weeks after exposure, and repeated inhalations of high doses of spores in dust from moldy hay, but not lower doses of spores, caused changes suggestive of AA after five
ACKNOWLEDGMENTS: The authors thank Dr. Urban Palmgren; Göran Blomquist, Ph.D; and laboratory technicians Maria Eriksson and Sven Olof Westermark for collecting and enumerating spore samples. We also thank Katrin Karlsson who evaluated spore samples with electron microscopy and Lars Belin, M.D., who performed the analysis of precipitating antibodies. Finally, we thank all physicians who reported cases to the project, notably the physicians of the Farmers Safety and Health Association.

REFERENCES

6 May JJ, Stallones L, Darrow D, Pratt DS. Organic dust toxicity (pulmonary mycotoxicosis) associated with silo unloading. Thorax 1986; 41:919-23.
34 Riesenfeld OI, Wolpe S, Garcia BJ, Hoffmann MK, Tuomanen E. Production of interleukin-1 but not tumor necrosis factor by human monocytes stimulated with pneumococcal cell surface.
components. Infect Immun 1989; 57:1890-93