

# Occupational Asthma to the Slime Mold *Dictyostelium discoideum*

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*Dictyostelium discoideum* is a slime mold that exists in a unicellular amoeboid form under certain nutritional conditions. In this form, it produces unique lysosomal enzymes that are valuable in studying cell-to-cell signaling systems. We report on a research microbiologist who developed rhinoconjunctivitis and asthma after release of *D. discoideum* from a pressurized canister. Immediate skin test reactivity was demonstrated to whole and lysed organisms. Enzyme-linked immunosorbent assay results revealed IgE antibody against *D. discoideum* whole organism, lysed organism, and lysosomal enzymes with the strongest response being directed toward lysosomal enzymes. Pulmonary function testing showed a decline in forced expiratory volume in 1 second and forced expiratory flow after modified laboratory exposure to *D. discoideum*. This case represents the first report of occupational rhinoconjunctivitis and asthma from slime mold.

The environment in which one works is increasingly recognized as a source of exposure to materials capable of causing upper and lower respiratory illness. Upper airway symptoms may include nasal congestion, rhinorrhea, sneezing, ocular itching, and lacrimation, whereas occupational asthma may manifest as cough, wheezing, chest tightness, or shortness of breath. International figures have reported an incidence of occupational asthma varying between 2 and 15%.<sup>1,2</sup>

Respiratory symptoms at the workplace may occur by toxic, irritant, or immunologic mechanisms. IgE-mediated occupational disease has been reported to a variety of compounds which can be classified by their molecular weight. Low-molecular-weight compounds, nonsensitizing in their own right, may become immunogenic after haptening with protein (eg, human serum albumin). Examples include isocyanates,<sup>3,4</sup> acid anhydrides,<sup>5,6</sup> antibiotics,<sup>7</sup> and platinum.<sup>8</sup> High-molecular-weight proteins can directly induce production of antigen-specific IgE in exposed workers. The protein may be of plant (eg, grain dust,<sup>9</sup> wheat flour,<sup>10</sup> vegetable gums,<sup>11</sup> enzymes<sup>12</sup>) or animal (eg, cow,<sup>13</sup> silk-worm,<sup>14</sup> organ extracts<sup>15</sup>) origin. Laboratory animals are a common cause of occupational respiratory allergy occurring in an estimated 20 to 30% of workers.<sup>16,17</sup> Although affected workers usually are sensitized to rats, mice, cats, and dogs, some workers develop novel sensitivities to proteins of microorganisms.

*Dictyostelium discoideum* is a slime mold (class Acrasiomycetes) that exists in a unicellular amoeba form when nutritional conditions are favorable, producing novel lysosomal enzymes. Upon removal of food sources, intercellular signals produced and rec-

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ognized by the amoeba cause cell aggregation, resulting in the multicellular slug stage. Further morphologic changes and cell invaginations give rise to the fruiting body capable of sporulation.<sup>18</sup> Because of *D. discoideum*'s unique developmental cycle, it is an excellent organism in which to study cell-to-cell signaling systems (eg, the cAMP system) in an attempt to unravel the mechanism by which growth and differentiation are initiated and regulated.<sup>19</sup>

We report on the development of IgE-mediated rhinoconjunctivitis and asthma from *D. discoideum* resulting from an occupational exposure.

## Case History

The patient is a 42-year-old non-smoking male laboratory worker evaluated for increased symptoms of rhinoconjunctivitis and asthma. Whereas the patient previously had experienced only seasonal symptoms and mild exercise-induced bronchospasm, he was now having symptoms during the winter. Further questioning revealed the development of itchy, watery, swollen eyes, sneezing, rhinorrhea, nasal congestion, chest tightness, and wheezing 15 to 30 minutes after removal of the *D. discoideum* from a pressurized container. He had been working with the specific organism for the past 18 months. There was no history of exposure to laboratory animals, no recent upper respiratory tract infection, no evidence of chronic sinusitis, smoke, or irritant exposure, or other potential triggers for his increasing symptomatology. Before our evaluation, heavy exposure to *D. discoideum* had caused severe enough symptoms to require overuse of his inhaled  $\beta$ -agonist.

Evaluation included routine prick skin testing to seasonal and nonseasonal aeroallergens, revealing 4+ reactions to alternaria and ragweed. With the suspicion of occupational sensitivity, efforts were made to identify specific IgE to *D. discoideum*, and to demonstrate objective changes in pulmonary function associated with exposure to the organism. The empiric recommendation of environmental control (handling materials

under laminar flow hoods and the use of a respiratory mask) provided significant improvement in the patient's symptoms and reduced his need for bronchodilators.

## Materials and Methods

### Whole Organism Stock

*D. discoideum* was maintained in a growth medium containing yeast extract, proteose peptone, phosphate buffer (pH 6.4), and glucose. Each milliliter of solution contained 30 to 40  $\times 10^6$  cells with approximately 10.8 mg of protein per 10<sup>8</sup> cells.

### Lysed Organism Stock

This was prepared by placing volumes of whole organism stock into a Parr pump (pressurized canister) and exposing the contents to 500 psi of nitrogen pressure for 15 minutes.

### Lysosomal Enzyme Stock

Harvested cells (3  $\times 10^7$ /ml) were resuspended in 5 mmol/L sodium phosphate buffer, then incubated for 6 hours. After cells were removed by centrifugation, 26 g of DEAE-cellulose was added to the supernatant. All the  $\alpha$ -mannosidase and  $\beta$ -hexosaminidase activity (lysosomal enzymes) was bound to the resin. The resin was collected by filtration and washed with sodium phosphate buffer. Secreted macromolecules were eluted and the eluate was dialyzed and concentrated to a volume of 6.0 ml.

### Prick Skin Testing (PST)

For PST, stock solutions of whole and lysed organism were diluted with phenol-saline diluent out to concentrations of 1:10,000.

### Enzyme-Linked Immunosorbent Assay (ELISA)

Antigen for ELISA was prepared from stock and diluted out to 1:1000 with sodium carbonate coating buffer, pH 9.4. Antigen was added to each well of a 96-well microtiter plate. After overnight incubation at 4°C, plates were washed with bovine serum albumin-saline. Diluted patient or control sera was added to appropriate

wells, incubated for 90 minutes at room temperature, and then washed. Rabbit anti-human IgE 1:1000 was added, incubated, and washed, followed by sheep anti-rabbit IgG-alkaline phosphatase conjugate, 1:1000. After incubation and washing, phosphatase substrate was added to each well and incubated for 30 minutes. The reaction was stopped with 5 N NaOH, and the optical densities were read by spectrophotometry at 405 nm.

## Results

### Prick Skin Tests

PSTs were performed on the patient as well as on an atopic control (Table 1). The patient demonstrated positive wheal and flare reactions to 1:100 whole and 1:100 lysed organism. The control patient was nonreactive even at undiluted concentrations.

### ELISA

ELISA for specific IgE directed against *D. discoideum* whole organism demonstrated a 3.7-fold greater patient optical density (OD) compared with control (Fig. 1), suggesting the presence of antigen-specific IgE. In addition, specific IgE was detected to lysed organism (Fig. 2), with patient OD 5.8 times higher than control. The greatest OD difference was demonstrated against lysosomal enzyme; patient OD was 7.5 times that of control (Fig. 3).

### Pulmonary Function Tests (PFTs)

PFTs were performed on the patient on a Monday morning before his arrival at his laboratory and were measured on a calibrated Multispiro

TABLE 1  
Prick Skin Tests

|                            | Patient  | Control  |
|----------------------------|----------|----------|
| Whole <i>D. discoideum</i> |          |          |
| 1:100                      | 3+       | Negative |
| 1:10                       | ND*      | Negative |
| Lysed <i>D. discoideum</i> |          |          |
| 1:100                      | 3+       | Negative |
| 1:10                       | ND*      | Negative |
| Saline                     | Negative | Negative |
| Histamine                  | 3+       | 4+       |

ND, not determined.

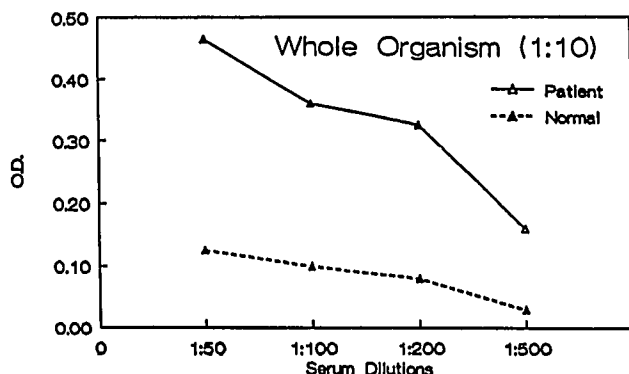


Fig. 1. OD versus serum dilution in ELISA for IgE-*D. discoideum* whole organism at 1:10. Patient OD is  $3.7 \times$  normal control pool OD at 1:50 serum dilution.

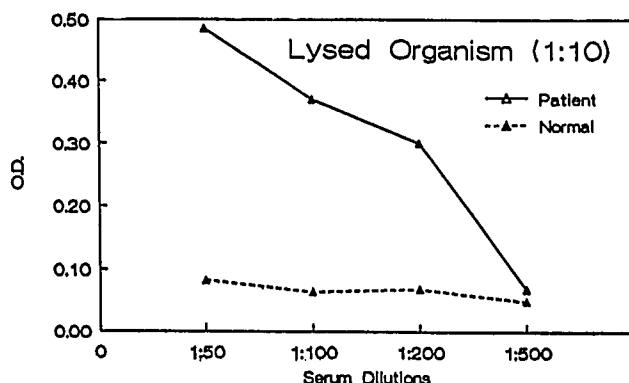


Fig. 2. OD versus serum dilution in ELISA for IgE-*D. discoideum* lysed organism at 1:10. Patient OD is  $5.8 \times$  normal control pool OD at 1:50 serum dilution.

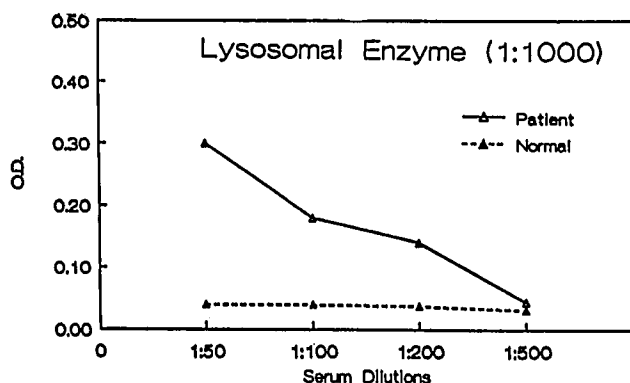


Fig. 3. OD versus serum dilution in ELISA for IgE-*D. discoideum* lysosomal enzyme at 1:1000. Patient OD is  $7.5 \times$  normal control pool OD at 1:50 serum dilution.

the same tasks without avoidance precautions.

## Discussion

While trying to analyze our patient's history to determine a cause of his increased symptoms, detailed questioning uncovered greater difficulties during the work week than on weekends. More intriguing was that symptoms of asthma and rhinoconjunctivitis would develop 15 to 30 minutes after venting the pressurized container used to lyse the *D. discoideum*, releasing organisms into the air. Suspecting the workplace as a source of our patient's clinical symptoms, the next step was to determine whether the response was on an immunologic or irritant basis.

Type I immediate hypersensitivity reactions are characterized by the presence of specific IgE and demonstrated in vivo by prick skin testing or in vitro by radioallergosorbent test or ELISA assays. PST and ELISA with the whole organism demonstrated a positive response, in contrast to our control. In addition, PST and ELISA with lysed *D. discoideum* demonstrated the presence of specific IgE, with the ELISA showing a greater difference in specific IgE (5.8 times) than even the whole organism (3.7 times). *D. discoideum* derives part of its uniqueness from its intercellular lysosomal enzymes.<sup>19</sup> Because our patient's symptoms developed upon exposure to the lysed organism and our ELISA demonstrated increased specific IgE to the lysed antigen, an ELISA to partially purified lysosomal enzyme was performed. A maximum 7.5-fold difference in patient OD readings versus control was demonstrated, suggesting that the patient's strongest response to antigen was against lysosomal enzyme.

It is not surprising that a known atopic person would develop specific

spirometer. The patient performed his usual routine, although modifying it by handling *D. discoideum* under a laminar flow hood whenever possible. Fifteen minutes after venting the pressure chamber, PFTs were repeated (Table 2). Forced expiratory volume in 1 second (FEV<sub>1</sub>) dropped 13.9% and the forced expiratory flow rate (FEF<sub>25-75</sub>) declined 30.7%. Administration of an inhaled  $\beta$ -agonist returned pulmonary function to nor-

mal. No reduction in air flow was observed in the PFT results of an atopic control subject who performed

TABLE 2  
Pulmonary Function Tests

|                      | Before <i>D. discoideum</i> | After <i>D. discoideum</i> | % Change |
|----------------------|-----------------------------|----------------------------|----------|
| FEV <sub>1</sub>     | 3.32 L/sec                  | 2.86 L/sec                 | -13.9%   |
| FEF <sub>25-75</sub> | 3.61 L/sec                  | 2.50 L/sec                 | -30.7%   |

No change was elicited in the atopic asthmatic control subject.

IgE antibodies to newly exposed allergens. Indeed, Davis found in his skin test results of asthmatics that more than half the males developed sensitization to at least two antigens,<sup>20</sup> and several studies have demonstrated a direct relation between atopic status and the development of occupational asthma including asthma due to *Bacillus subtilis*<sup>21</sup> and coffee bean dust.<sup>22</sup>

Although specific IgE to *D. discoideum* was apparent, a causal relationship to signs and symptoms required demonstration. The patient's symptoms were severe, requiring inhaled  $\beta$ -agonists every 1 to 2 hours, and would routinely develop within 15 to 30 minutes after venting the pressure chamber (used in lysing), with its associated release of contents into the air. Although a specific work-induced bronchial challenge would have been preferable, the patient's unwillingness to reexperience that degree of symptomatology necessitated an amended occupational challenge protocol. Despite having only a minor exposure to antigen, the patient still experienced sneezing, ocular itching, and conjunctival injection without the development of lower respiratory tract symptoms. Postexposure PFTs revealed 13.9% and 30.7% reductions in FEV<sub>1</sub> and FEF<sub>25-75</sub>, respectively. We believe that these changes in PFTs are quite notable in that no symptoms of asthma developed. Previously, the patient's heavier exposures were associated with marked symptoms and undoubtedly accompanied by greater reductions in pulmonary function. McFadden et al<sup>23</sup> and Rubinfeld and Pain<sup>24</sup> have shown significant reductions in FEV<sub>1</sub> while patients were asymptomatic. Thus, we believe that if FEV<sub>1</sub> decreased 13.9% without symptoms, pulmonary function changes with symptoms requiring frequent  $\beta$ -agonist use were most probably associated with greater reduction in airflow.

The criteria for documenting reversible airway obstruction in occupational asthma are not well defined. Whereas a decrease in FEV<sub>1</sub> of 20% would be considered more strongly positive, guidelines set forth by the American Academy of Allergy and Immunology Committee on Occupa-

tional Lung Disease give the criterion of a 10% or greater reduction in FEV<sub>1</sub> over a single work shift or 20% or greater reduction in peak flow over the course of a single day.<sup>25</sup>

Identifying the cause for a sudden change in the course of a patient's asthma can be a challenging problem. Occupational asthma was suspected by characteristic historical features and supported by PFTs. Although an irritant response could provoke bronchospasm in an asthmatic patient, the laboratory findings and associated clinical symptoms of allergic rhinoconjunctivitis led us to believe that this was a Type I hypersensitivity reaction. Specific IgE was identifiable by both in vivo and in vitro testing. *D. discoideum*'s lysosomal enzyme stimulated the largest specific IgE response when comparing patient versus control serum. Because symptoms developed after lysing of the organism, *D. discoideum*'s lysosomal enzyme may represent one of the main offending antigens. Cross-reactivity between epitopes from *Alternaria* (class Deuteromycetes) and *D. discoideum* (class Acrasiomycetes) is an intriguing possibility as a factor leading to the development of our patient's clinical sensitivity.

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