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Myxomycete (slime mold) spores: unrecognized aeroallergens?

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ABSTRACT

Background: Myxomycete spores are present in the outdoor air but have not been studied for allergenicity.
Objective: To determine whether patients with seasonal allergic rhinitis (SAR) symptoms are sensitized to myxomycete spores.
Methods: Myxomycete specimens were collected in the field. Nine species of myxomycetes were collected and identified: *Arcyria cinerea*, *Ceratiomyxa fruticulosa*, *Fuligo septica*, *Hemitrichia clavata*, *Lycogala epidendrum*, *Metatrichia vesparium*, *Stemonitis nigrescens*, *Tubifera ferruginosa*, and *Trichea favoginea*. Allergen extracts were made for each species. Protein content of each extract was measured by bicinchoninic acid assay. Protein electrophoresis was performed. Subjects with a history of SAR symptoms were enrolled, and allergy skin prick testing was performed with each extract.
Results: Protein content of the extracts ranged from 1.05 to 5.8 mg/mL. Protein bands were seen at 10 to 250 kD. Allergy prick testing was performed in 69 subjects; 42% of subjects had positive prick test results for at least 1 myxomycete extract, with 9% to 22% reacting to each extract. Five of the 12 subjects who tested negative for all allergens on the standard aeroallergen panel had positive prick test results for myxomycetes.
Conclusion: Forty-two percent of subjects with SAR were sensitized to myxomycete spores. A significant subset of subjects who had SAR symptoms and otherwise negative skin test results showed sensitization to myxomycetes. These spores are present in the outdoor air during the summer and autumn and might be significant aeroallergens.

Introduction

The commonly recognized outdoor aeroallergens include tree, grass, and weed pollens and fungal spores. Patients with spring and autumn exacerbations of their allergic rhinitis symptoms are typically allergic to pollens, and patients with symptoms during wet weather and in the late autumn usually have fungal spore allergies. However, it is not unusual for a patient to present with symptoms typical of seasonal allergic rhinitis (SAR), exacerbated by outdoor exposures and improving with allergy medications, yet with no positive allergy skin test results. In these cases, it seems likely that the patient is reacting to an unidentified outdoor aeroallergen. Myxomycetes are a potential source of allergenic spores in the outdoor air.

Myxomycetes, popularly known as *slime molds*, are eukaryotes that have a complex life cycle, with phases typical of animal and fungal organisms. In the plasmodial (slime) phase, the organism moves slowly along its substrate, ingesting bacteria. When the supply of nutrients or water becomes low, the plasmodium morphs into a fungus-like fruiting body and makes spores similar to those of fungi (Fig 1).[1] The fruiting bodies of myxomycetes vary in size from microscopic to quite large, and the number of spores produced per fruiting body varies correspondingly. At one extreme is the well-known "dog vomit slime mold" (*Fuligo septica*), which appears on mulch beds and decaying logs in the summer, with the plasmodial phase resembling its namesake. The fruiting bodies of *F septica* can reach up to 12 inches or larger in diameter and are packed with millions of spores. Many other species are smaller but very numerous.

The air sampling data for the author's area is performed using a rotorod impaction sampler, which undersamples small spores; nevertheless, many myxomycete spores are identified, especially during the summer and autumn (Fig 2). A longitudinal birth cohort study found myxomycete spores in 59% of the indoor and 80% of the outdoor air samples.[2] Thus, myxomycete spores are potential aeroallergens that have not yet been investigated, except for a study of basidiomycete spore allergies that included intradermal skin testing with *F septica*,[3] and an occupational asthma case report related to *Dictyostelium discoideum*.[4]

The purpose of this study was to determine whether patients with SAR symptoms are sensitized to myxomycetes, and whether myxomycete allergy might account for some cases of SAR with negative aeroallergen skin test results.

Methods

Myxomycete Collection and Identification

Myxomycete fruiting bodies were located in local woods, parks, and landscaping mulch beds and collected. They were identified

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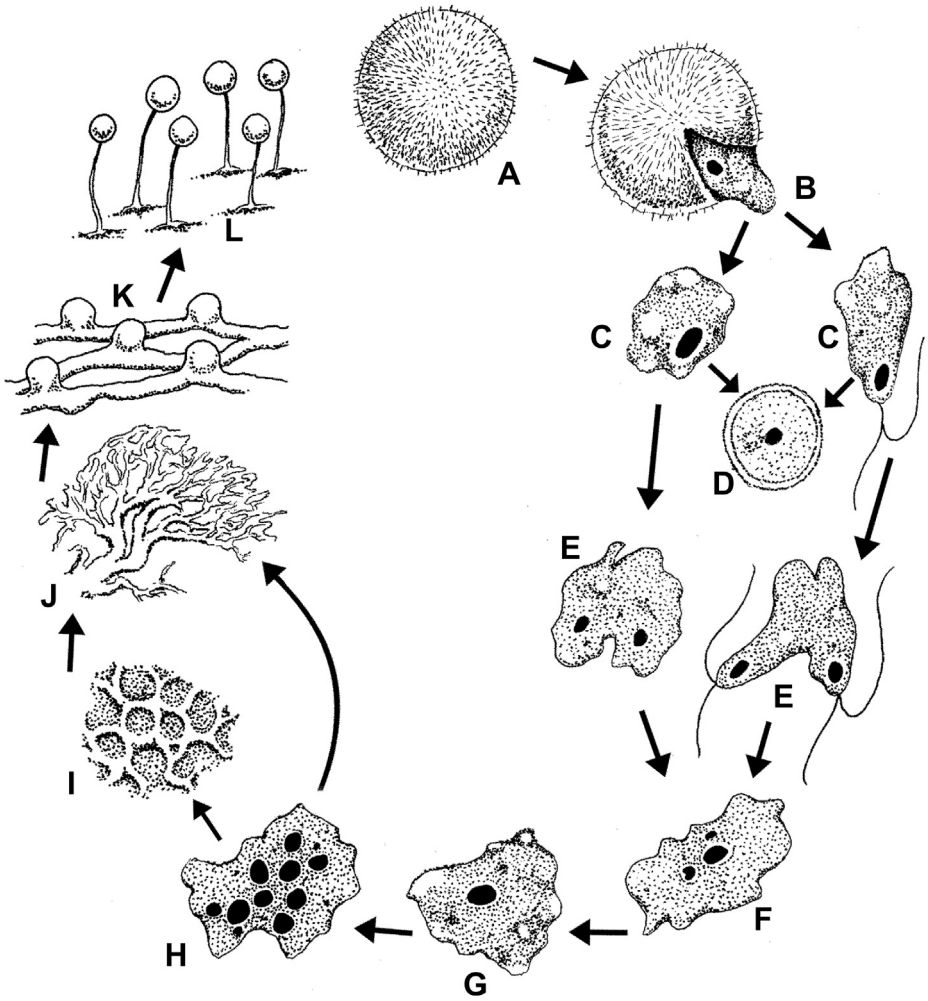

Figure 1. Generalized life cycle of the myxomycetes. *(A, B)* A protoplast emerges from the spore. *(C)* The protoplast can take the form of an amoeba or a flagellated cell during the first trophic stage. *(D)* Under dry conditions or in the absence of food, amoebae form a microcyst, or resting stage. *(E, F)* Compatible ameboflagellates fuse to form a zygote *(G)*. *(H)* The nucleus of the zygote divides by mitosis and each subsequent nucleus also divides without being followed by cytokinesis, thus producing a single large cell *(J)*, the plasmodium, that represents the second trophic stage. Under adverse conditions, the plasmodium can form the second type of resting stage found in myxomycetes, the sclerotium *(I)*. *(K, L)* Fruiting bodies are formed from the plasmodium. During formation of the fruiting bodies, spores are produced. Adapted from Stephenson[1] with permission of the author.

using a field guide by Stephenson and Stempen[5] and by the Eumycetoan Project Web page (http://slimemold.uark.edu/martin.htm) using macroscopic and microscopic features. A sample of each species was sent to Steven L. Stephenson, PhD, at the University of Arkansas, to confirm the identity. The spores were collected from species with a contained spore mass (*Lycogala epidendrum, F septica*) by dissecting the spore mass from the fruiting body. Capillitial elements were present in the spore mass. In other species *(Arcyria cinerea, Ceratiomyxa fruticulosa, Hemitrichia clavata, Metatrichia vesparium, Stemonitis nigrescens, Trichia favoginea, Tubifera ferruginosa;* eFigs 1-18), the spores were inseparable from the tiny sporangia, which were collected whole. Thus, extracts for these species included spores, capillitia, peridia, and stalks. The material was stored under desiccation until a sufficient quantity had been collected for extraction.

Extraction

The extraction method described by Liengswangwong et al[6] and Horner et al[7] for basidiospore extracts was attempted, but failed. This method involves extracting the spores in ammonium bicarbonate solution, centrifuging and filtering to remove the spores, lyophilizing the extract, and resuspending the lyophilate in phosphate buffered saline (PBS) and glycerin. Most myxomycete lyophilates contained insoluble crystals and could not be successfully dissolved into the PBS. Therefore, the following extraction procedure, which is similar to that used for the production of commercial fungal extracts, was followed:

1. For acetone defatting, 1,500 mg of each specimen was mixed with acetone and shaken gently for 15 minutes. The mixture was centrifuged at 4,000 rpm for 30 minutes, and then the acetone supernatant was poured off. The myxomycete material was dried in a glass beaker under a hood.
2. The dried myxomycete material was suspended in a solution of 50% glycerin in PBS (pH 7) at 1:10 wt/vol (ie, 1 g of material per 10-mL solution). The mixture was intermittently shaken to keep the material homogeneously suspended in the buffer solution. The purity of the sample was assessed by placing a drop of the extract on a microscope slide and counting all elements present up to 100 particles at 400× magnification. Acceptable purity was defined as less than or equal to 1% foreign spores and less than 3% soil or bark debris.
3. The material was kept in PBS and glycerin for 48 hours at room temperature, and then the suspension was centrifuged at 7,000 *g*