

Mold allergy — spores and mycelium as allergen sources

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The purpose of the present paper is to discuss what parts of moulds are important as sources of allergens.

Any member of the taxonomic kingdom Mycetozoa are defined as fungi. Moulds are filamentous fungi. They are a group of eukaryotic, spore-bearing organisms (1, 4, 6). The basic structural unit in most moulds is the hyphae which is a tubular, branching thread. Hyphae may be divided by cross walls, called septa, to form compartments or cells. They may lack cross walls, in which case they resemble an open continuous tube with nuclei floating freely within the cytoplasm. The septa are rarely solid. Typically one or more pores are present in each septum so that there is cytoplasmic continuity between adjacent hyphal cells. The hyphae usually possess a definite cell wall composed of chitin and other complex carbohydrates. Collectively, all hyphae of one fungal organism are called a mycelium. Mycelium may grow on surfaces (several fungi imperfecti) or in the soil (several Basidiomycetes).

Fungi are capable of living under a variety of environmental conditions. The appearance, as well as the antigenic and biochemical composition of the mycelium must be expected to vary considerably. It should also be mentioned that many fungi with a single genotype may produce two or more phenotypes (pleomorphism). It is often impossible to predict what will occur in a particular species, and one form or spore-producing phase may metamorphose into another.

A spore is a microscopic reproductive unit. It is a fragment of an organism consisting of a minimum complement of essential cellular components, including at least one nucleus. Enclosing the cytoplasm is a unit membrane and one or more cell wall layers of complex carbohydrate nature. Then follows a layer of a hydrophobic fibrous new-work of wall components, referred to as »rodlets» (4). Surrounding the rodlet layer may be an additional amorphous layer. The spore represent a stable state of the fungus without metabolism which can survive under unfavourable conditions for prolonged periods of time. Its purpose is to allow spreading of the mould as well as to restart growth when conditions again are favourable.

A group of moulds that have allergenic properties, particularly well documented in the New Orleans area, are the Basidiomycetes. These are typically the common mushrooms with underground mycelium and visible overground fruitbodies which may contain large amounts of spores. The Basidiomycetes represent an interesting model for the question as to what parts of the moulds are the naturally sensitizing aeroallergen. It is clearly the spores of the Basidiomycetes which function as aeroallergens. The mycelium is underground and not likely to be spread, and in many cases the fruitbodies are never considered for eating. It is therefore particularly interesting that mycelial extracts of Basidiomycetes are allergenic in man in that they may elicit positive skin reactions in sensi-

tive individuals as demonstrated by Lopez and coworkers in New Orleans (8). This was verified by Lehrer and Aukrust, who obtained positive RAST-results both with individual extracts of Basidiomycetes mycelium, and of spores (unpublished results). This suggest that mycelium and spores of Basidiomycetes contain common molecules or determinants which may react with specific IgE antibodies. It does not, however, prove that mycelium is fully representative of allergens present in spores.

The allergenically most important fungi, however, all belong to the formclass fungi imperfecti. This form-class is defined by an appearant lack of sexual reproduction and these fungi are only present as mycelium or spores (called conidia). Examples are fungi in the genera *Alternaria*, *Cladosporium*, *Penicillium* and *Aspergillus*. The fungi imperfecti conidia are developed from the mycelium and must be expected to have a more constant composition than the often variable mycelium. The major part of this paper is related to a discussion on the conidia or mycelium of the fungi imperfecti as allergen sources.

With respect to fungi imperfecti, air sampling has shown that mycelial fragments may be distributed in the air although such fragments seem to comprise less than 10 % of the particles of relevant size – the rest being spores (conidia). It is likely that the conidia are the dominating natural sensitizing agent as well as being the major source of symptoms-giving allergens. On the other hand many commercially available extracts of fungi imperfecti are made from mycelium with little or no spores present (6, 7). Such extracts would theoretically be adequate if mycelium contains the same allergenic molecules as the conidia, but would be absolutely inadequate if not. For this reason, comparison of antigenic and allergenic composition of mycelium and spores of various fungi imperfecti is important. It is particularly important since it seems difficult to obtain pure spore preparations in sufficient amounts at a reason-

able cost for commercial extract preparation. Studies of spores versus mycelial allergens are long overdue, but it is a depressing fact that this problem has largely been ignored. To our knowledge, the paper by Hoffman and coworkers from 1981 (5) is the only study which adequately addresses this question. These authors obtained 90–96 % pure spore preparations and 97 % pure mycelium preparation of an *Alternaria alternata* strain and compared them with a series of biochemical and immunochemical techniques. Their spore extracts were clearly distinct from their mycelium extract, although the spore extracts contained many similar or identical antigens to the mycelium extract. The spore extracts contained eight antigens not found in the mycelium extract, but mycelium did not contain any antigens not found in the spores. Quantitative analysis of the spore and mycelium extracts gave quite different results. About half of the patients studied were more reactive to spore derived allergens than to those allergens found in both mycelium and spores. Some patients, on the other hand, were clearly most reactive to allergens present both in the mycelium and the spores.

Similar experiments have recently been performed in our laboratories using individual spore and mycelium preparations of one *Alternaria alternata* strain. Both the extracts were coupled to RAST discs. RAST-inhibition (11) experiments were performed using these two sets of discs and a pool of ten *Alternaria* sensitive patient sera (Fig. 1). Both preparations could inhibit both types of discs to a degree of more than 90 %. This may indicate that the major IgE-binding material, as measured by RAST, were located both in the spore- and the mycelium-preparations. Using the spore extract discs (Fig. 1.a), the mycelium extract showed somewhat different dose-response relation than that obtained with the spore extract. The inhibition curves were not parallel and there was a tendency of the mycelium extract curve to reach a plateau below 100 % inhibition.

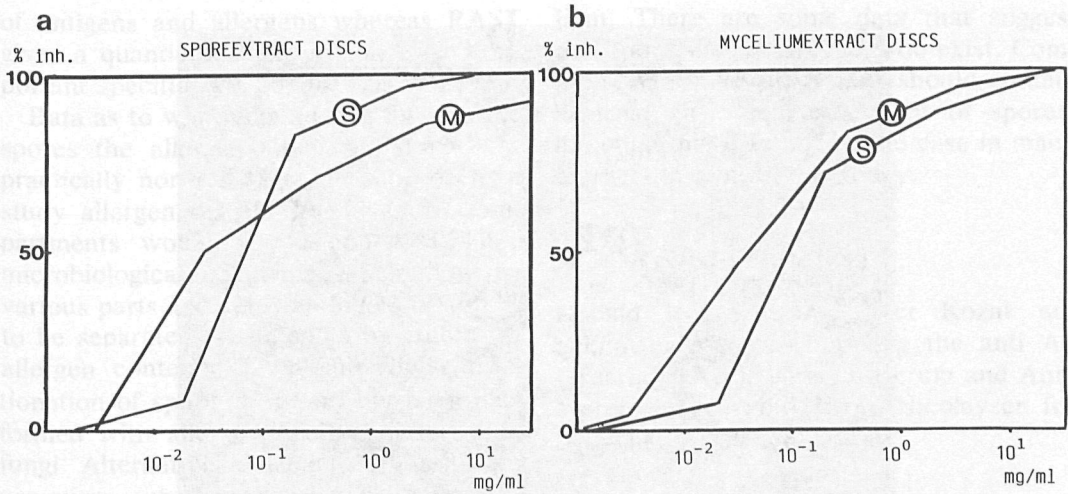


Fig. 1. RAST-inhibition (*Alternaria alternata*).

RAST-inhibition dose-response relation of both (spore(s) – and mycelium (M) -extract against (a) discs carrying the spore extract and (b) discs carrying the mycelium extract using a patient serum pool.

Using discs carrying the mycelium extract (Fig. 1.b), however, the inhibition curves had similar shapes both given close to 100 % inhibition. These results may indicate that the spore extract was representative of the mycelium extract with respect to total allergenic content, as measured with the patient serum pool used. On the other hand, the mycelium extract may lack or have low concentration of some allergens present in the spore preparation. Crossed immunoelectrophoresis (2) was performed using a broad spectered anti*Alternaria* rabbit antiserum containing antibodies both to spores and mycelium (Fig. 2). A spore-specific antiserum was included in the intermediate gel. Both the spore and the mycelium preparations were run in separate experiments. The plates were later subjected to serum incubation with the patient serum pool, followed by incubation with ^{125} I-anti-IgE and autoradiography (2) (Fig. 2).

Rocket immunoelectrophoresis of the spores and mycelium preparation were performed using monospecific antiserum against the major allergen, Alt-1 (3). The mycelium preparation contained approximately 3 times as much of Alt-1 as did the

spore preparation (results not shown). This may explain the somewhat higher potency found with the mycelium extract in the RAST-inhibition experiments (Fig. 1).

The immunoelectrophoretic experiments showed that the spore preparation seemed to be more heterogeneous and contained a higher number of both antigens and allergens than did the mycelium preparation. In fact, our results confirmed those of Hoffman and coworkers in that there seemed to be spore specific antigens and allergens that could not be detected in the mycelium preparation in *Alternaria alternata*.

Such studies, however, should be repeated in different laboratories with different species and strains. A complicating factor is the tendency of moulds to vary (10). An observed difference between a spore preparation and a mycelium preparation may be due to a real difference between mycelium and spores. It may also, however, be due to the fact that with the present technology they necessarily represent two different preparations, and that other results may be obtained if repeated with additional spore and mycelium preparations of the same strain. The possibility of cross

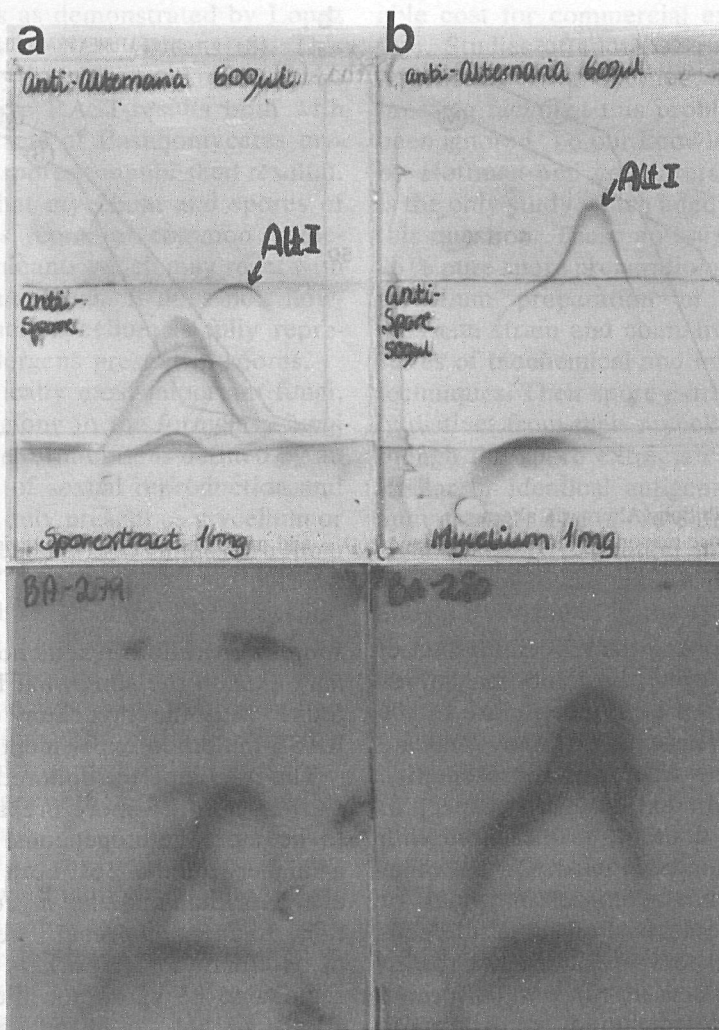


Fig. 2. Crossed immunoelectrophoresis of 1 mg spore extract (a) and 1 mg mycelium extract (b) of *Alternaria alternata*. Rabbit anti-spore extract was incorporated in the intermediate gel and a broad spectered rabbit antiserum pool to both spores and mycelium was included in the upper gel. Corresponding X-ray films from autoradiography experiment of the same plates are shown below.

contamination of the spore and mycelia extracts must always be considered. This phenomenon may also influence the specificities of the rabbit antisera used. The rabbit immune system may function as an amplification system and a so called »spore specific antiserum« may contain antibodies to contaminating mycelial fragments. The antisera may even lack precipitating antibodies to some antigens. Only limited

conclusions can be drawn from experiments using such reagents.

A reservation should also be made with respect to RAST-results since they only reflect allergens sufficiently present on the discs and the major IgE specificities of the sera used. On the other hand the combined use of the different techniques are advantageous — the immunoelectrophoretic experiments give a high resolution

of antigens and allergens whereas RAST gives a quantitative expression of the important specific IgE populations.

Data as to what compartment within the spores the allergens may be found, are practically non-existent. One approach to study allergen distribution in spore compartments would be to apply available microbiological techniques which allow the various parts and cell wall layers of spores to be separated (4) followed by studies of allergen contents. Such subcellular fractionation of spores has yet only been performed with allergologically uninteresting fungi. Alternatively, it may be possible to use microscope techniques based on cross-sectioning combined with fluorescent or radioactively labelled antibodies.

Most spores are trapped in the bronchial tree and removed by ciliary movement over to the gastrointestinal tract after a few hours. One may therefore speculate that antigens secreted or extracted *in vivo* under physiological conditions are the important allergens. However, this may be an oversimplification since little is known of the effects of the presence of biochemically and immunologically active cells, such as macrophages, in the bronchial lumen. The hydrophobic rodlet layer of the spores may restrict extraction of hydrophilic antigens. Small spores, such as those of *Aspergillus*, may be trapped as far down as in the alveolea. They may remain there for long periods of time and may finally be removed by phagocytosis since the alveolea do not contain cilia. On the other hand the alveolea contain surfactants that may enhance extraction through the hydrophobic rodlet layer. It is an interesting observation that some fungal species known to give delayed hypersensitivity reactions in man, such as *Aspergillus*, have spores which are likely to reach the alveolea (4).

In conclusion, the spores seem to be the natural sensitizing part of fungi. The mycelium may contain many of the same allergenic molecules or determinants since the spores are developed from the myce-

lium. There are some data that suggest that spore specific allergens do exist. Commercial extracts of moulds should contain at least an adequate amount of spores, although this may not be the case in many extracts in clinical use today.

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