# MOLDS, AGENTS AND DISEASE

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## Abstract

Molds contain or produce a variety of biologically active agents such as the cell wall component (1→3)-β-D-glucan, extracellular polysaccharides and mycotoxins. Exposure to molds has been related to airways inflammation, hypersensitivity pneumonitis and reduced resistance to infection. As the exposure under field conditions comprises a multitude of agents, which often covary, it is difficult to attach a specific agent to a specific disease. (1→3)-β-D-glucan has been used as a marker of mold biomass and risk for airways inflammation in studies on indoor environments.

## Background

Molds are ubiquitous in man's environment. Like many other environmental agents, they are tolerated without adverse effects if the concentration is natural. When climatic conditions or handling of moldy materials increase their number, serious disease may develop. The first recorded warnings against molds are found in the Bible, where a caution against what was probably mold in houses is found in the book of Leviticus (14th chapter, verses 34-47).

There is general agreement that the determination of viable organisms is not a good estimate of the exposure or the risk. This is understandable as the effects, as we shall see, are not of an infectious nature which would require living organisms – but of a toxic/inflammagenic nature, where the dose is determined by the biomass. A consequence of this is the question if there are specific agents in molds that cause disease.

The purpose of this paper is to review some major specific agents in molds, the diseases that have been described in connection with mold exposure and how the two relate to one another.

## Agents in Molds

Mold is a layman's name for a mixture of various species of fungi, which grow in humid conditions on suitable surfaces. There is a large variation in species, depending on the growth conditions, particularly humidity, temperature, and surface properties. In real life conditions, there is also bacterial growth in connection with molds; the number of bacteria may indeed be larger than the number of fungi. Molds contain a number of specific agents. Table 1 lists the most commonly investigated such agents.

### Structural Agents

#### Ergosterol

Ergosterol is a sterol present in the fungal cell wall and has been used to estimate fungal biomass. Relationships have been found with the extent of symptoms or spirometry, indicating a role for fungi in these symptoms [Miller *et al* 1988]. It is not believed that ergosterol as such is a causative agent. It can, however, be used as a surrogate for exposure to fungal biomass or to the specific agents therein.

#### Extracellular Polysaccharides

Extracellular polysaccharides (EPS) are heat stable water-soluble, non-branched glycoproteins and are part of the mycelial cell wall. EPS are released into the environment during growth. They have antigenic properties that are usually specific on the genus level. The quantity of EPS is relatively well related to the quantity of mycelium and it can thus be used as a marker for fungal biomass. There is no evidence for a pathogenic role for EPS in fungal related disease.

#### (1→3)-β-D-Glucan

Cell wall of plants, bacteria and fungi contain a polyglucose compound - (1→3)-β-D-glucan - in the cell wall. These polymers can exist as a single polymer strand with a helical conformation (single helix) or as a stable complex of three polymer strands forming a triple helix.

There is an abundant literature on the biological effects of (1→3)-β-D-glucans. Their ability to enhance the function of macrophages, neutrophils and other inflammatory cells has been well documented [Di Luzio 1985; Williams 1997]. They have been shown to enhance humoral and cell-mediated immunity to antigens. In addition, they exert additive or synergistic effects on immunity when combined with a variety of agents. (1→3)-β-D-glucan can also induce inflammation. Macrophages secrete inflammatory cytokines after exposure to different (1→3)-β-D-glucans *in vitro* [Ohno *et al* 1995].

The effects found after inhalation at dose levels close to those in the environment are different from those found in *in vitro* models. The findings from three inhalation exposures illustrate the mode of action of (1→3)-β-D-glucan. After an acute inhalation, there is no invasion of neutrophils in the airways as is the case after exposure to endotoxin [Fogelmark *et al* 1992]. If an exposure to (1→3)-β-D-glucan preceded the exposure to endotoxin, a dose dependent depression of the endotoxin induced neutrophil response was found.

After a five weeks exposure in guinea pigs, there was an increase in the number of lymphocytes in the airways, if

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endotoxin was administered together with (1→3)-β-D-glucan, but not after exposure to (1→3)-β-D-glucan only [Fogelmark *et al* 1994]. There was also an increase in the number of subepithelial eosinophils.

Inhalation of the antigen ovalbumin (OVA) caused a very small increase in the amount of circulating IgG antibodies after five weeks exposure [Rylander and Holt 1998]. If endotoxin was administered simultaneously, there was a massive increase in antibody titers. This response was abolished by the simultaneous inhalation of (1→3)-β-D-glucan. In the same experiment, the eosinophilia induced by the OVA exposure was suppressed by the simultaneous exposure to (1→3)-β-D-glucan.

The data from inhalation studies using pure (1→3)-β-D-glucan thus suggest that effects after inhalation are not agent-effect related but rather appear in form of a priming, where (1→3)-β-D-glucan alters the reaction to a following exposure, be this an inflammatory agent or an antigen. The effects are found after very low levels of exposure, in the order of picograms/kg body weight.

**Others**

A number of other cell wall products such as muramyl peptides, arabinogalactan and mycolic acids are known to be toxic. No data are available on the concentration of these agents in different environments or their effects after inhalation exposures.

## Metabolic Agents

**Enzymes**

Fungi produce extracellular enzymes and some fungi retain these in their cell wall. Enzymes from bacteria are well known inducers of hyperreactivity and exposure to *Bacillus subtilis* proteases in industrial settings is known to cause asthma. Alpha-amylases from Aspergillus species and cellulases from Tricoderma increase the risk for allergies among bakers. Enzymes from bacteria in the gut flora are believed to be the causative agent in house dust mite induced sensitivity. Information on airborne concentrations of fungal enzymes is scanty – for cellobiohydrolase concentrations of 40-60 ng/m$^3$ and 6-7 µg/m$^3$ have been reported at different workplaces. Dose-response data are not available.

**Volatile Hydrocarbons**

During growth, molds emit several volatile agents (MVOC) of which a number has been identified [Sunesson *et al* 1995]. The emission of MVOC requires living colonies of the organism and is no measure of biomass.

Several of the MVOCs are irritating and have been shown to cause airways inflammation in rodents when inhaled in high amounts. So far, no field studies on the relation between effects and MVOC have been reported. Human inhalation studies of the agents in environment related levels have not been published.

**Toxins**

Mold toxins are non-volatile, low molecular weight secondary metabolites. Sources within the fungus are the mycelium, the extracellular matrix or the spores. Probably all fungi produce one or several different kinds of mycotoxins, provided that growth conditions are suitable.

A common property to mycotoxins is to interfere with the cell metabolic processes and a single toxin can thus have several end effects in humans depending upon where the interference takes place. In humans mycotoxins are carcinogenic, neurotoxic, and teratogenic. These effects probably require a metabolism for instance via the P450 system in the lungs, the liver or the gut.

Ingestion is by far the most common exposure route where effects in humans have been described. Most of the few cases of possible airborne exposure to mycotoxins are among farm workers, persons processing moldy materials and in homes with excessive mold growth.

Mycotoxin contamination has been demonstrated in homes containing *Aspergillus fumigatus* [Miller *et al.* 1988]. Mycotoxin-producing fungi have been found in residential buildings. Smith *et al.* [1992] identified mycotoxins in 83 fungal isolates from damp buildings in Edinburgh. Evidence of cytotoxic materials was present in 47% of the samples.

Pulmonary hemorrhage and nose bleeding has been associated with exposure to *Stachybotrus chartarium* which produces a powerful mycoto xin [Etzel *et al* 1998]. In a study on childcare centers and schools in Finland, a new toxin, probably of fungal origin, was identified, using an *in vitro* toxicity test on extracts from wall materials in the buildings [Anderson *et al* 1997].

A major difficulty regarding mycotoxins is the lack of suitable methods for field measurements. The identification of an organism and demonstration that it is capable of producing toxin when grown in the laboratory is not a proof of causality.

## Diseases Related to Mold Exposure

Effects caused by molds were first reported by Floyer [1726] who described an asthmatic reaction after the inhalation of molds. About 150 years later, Blackley [1873] described his own reactions after inhaling Penicillium spores:

> "*The spores of the microscopic fungi, I have reason to believe, will, when brought into contact with the respiratory mucous membrane, generate symptoms not unlike those of hay fever in some respects but*

*differing materially in others – being much like those of ordinary influenza.*"

Blackley, who had suffered from hay fever since childhood, a few days later developed a severe attack of hoarseness leading to complete aphonia that lasted for about two days and ended with a "bronchial catarrh." Similar reactions after inhaling large amount of molds have later been reported by Gravesen [unpublished].

Table 2 lists the different diseases that have been related to excessive exposure to molds in occupational and general environments.

**Pulmonary Effects**

Airways inflammation (bronchitis, reactive airways disease syndrome (RADS), non-allergic asthma) is characterized by irritation in the airways, stuffy or swollen nose, increased airway responsiveness and dry cough. Spirometry, however, is often normal and the clinical criteria for asthma are not met. Mild stages of airways inflammation show only symptoms of airways irritation and in more advanced stages there is a feeling of continuously swollen nose, cough and tiredness. Airway responsiveness as measured with a methacholine or histamine test is increased and, in advanced stages, there may be a ventilatory impairment in terms of decreased $FEV_1$.

Airways inflammation is a non-specific reaction that may be caused by several different environmental agents. It is not an allergic response, and tests for antibodies to specific agents are negative. It has been debated whether airways inflammation is a disease or merely a symptom. In view of the discomfort and reduced work capacity it may cause, as well as the clear relation to specific environmental exposures, it seems reasonable to delineate it as a disease.

There are a large number of field studies where airways inflammation has been related to mold exposure. Some of these studies have used (1→3)-β-D-glucan as a marker of fungal biomass.

One investigation was performed in a number of buildings where symptoms related to indoor air had been reported [Rylander *et al* 1992]. A building with no symptoms served as a control. A questionnaire was distributed to 405 persons and measurements of (1→3)-β-D-glucan were made in the buildings. The proportion of persons with nasal and throat irritation, dry cough, headache and excessive tiredness was higher in the buildings with more glucan in a dose dependent fashion.

A case study in Switzerland comprised a clinical investigation of two boys living in a house with indoor mould growth and airborne (1→3)-β-D-glucan levels ranging between 5 and 106 ng/m$^3$ (mean 41.9) [Rylander *et al* 1994]. The boys developed severe airway symptoms in terms of cough, wheezing and tiredness after about 6 months living in the house. They finally moved out of the house and the symptoms disappeared. About a year later, the parents developed similar symptoms and had to move out of the house.

Some field studies on airways inflammation relate to the school environment. In an investigation in two schools – one with mold problems and another without problems, the extent of respiratory symptoms was significantly higher in the school with higher levels of (1→3)-β-D-glucan (15.3 ng/m$^3$) as compared to the school with low levels (2.9 ng/m$^3$) [Rylander *et al* 1998]. Among atopic children, the extent of symptoms of dry cough, cough with phlegm and hoarseness was similar to the non-atopics in the low (1→3)-β-D-glucan school but significantly more common in the high (1→3)-β-D-glucan school. A study from the Netherlands recently reported a relation between decreases in spirometry in children and the amount of (1→3)-β-D-glucan in the house dust [Douwes personal communication].

***Hypersensitivity pneumonitis*** (allergic alveolitis, granulomatous pneumonitis) is an inflammatory disease that is characterized by a lymphocyte infiltration in the lung parenchyma which may proceed to granuloma and even fibrosis. Agents that cause hypersensitivity pneumonitis do so by influencing the activity of T cell lymphocytes, probably the function of suppressor cells [Schuyler *et al*. 1994]. There is now general agreement that it is not a type III disease. The disease probably has a subclinical phase, characterized by a lymphocyte infiltration in the lung parenchyma and airways where small granuloma may form but only visible under the microscope in bronchial biopsies.

The most widely recognized causative agent is molds. It is of interest that (1→3)-β-D-glucan causes granuloma in parenchymal tissue, including the lung, after injection and a role for this agent in hypersensitivity pneumonitis has been suggested [Fogelmark *et al.* 1994]. A hallmark for hypersensitivity pneumonitis is a change in CD4+/CD8+ ratio. Exposure to mold in home environments also caused shifts in the CD4+/CD8+ ratio among children [Dales *et al* 1998].

***Reduced resistance to infection*** has been seen among children exposed to high concentrations of molds indoors. In a study from Geneva, 304 children were investigated using a questionnaire [Rylander and Mégevand 1999]. There was an increased risk for colds and otitis related to dampness at home or mold at home. No increased risk was seen for the keeping of pets or parents´ smoking habits. A reduction of the risk for infection was seen in a Finish school after the building has been cleaned for mold growth [Koskinen *et al* 1997].

***Allergy*** to molds is well known and there are a number of specific allergens known (Gravesen 1979]. The incidence

of the disease is quite low in a population sample and at workplaces most persons with a true allergy would have left the workplace.

**Systemic Effects**

***Toxic pneumonitis*** (inhalation fever, organic dust toxic syndrome) is characterized by an increase in body temperature, shivering and muscular and joint pains, and symptoms resembling influenza. There is blood neutrophilia, and markers for inflammation such as eosinophilic cationic protein (ECP) are increased. The disease is short-lived and the symptoms disappear within a few days [Von Essen *et al*. 1990]. Indoors, toxic pneumonitis occurs mainly in connection with humidifiers. It may also appear after very dusty work, such as cleaning dirty shelves, sweeping dirty floors and changing wall-to-wall carpets. The number of cases in an exposed population can be close to 100%. As the disease is not of allergic nature, it is closely related to dose levels with a threshold that may, however, be different between sensitive and non-sensitive individuals.

Endotoxin is a causative agent of toxic pneumonitis, and a guideline for this effect has been proposed as 200 ng/m$^3$ [Rylander 1997]. Ever since the first description of "mycotoxicosis" [Emanuel *et al* 1975], it has been debated whether fungal spores could cause the disease. Data from human challenges are not available but results from animal experiments demonstrate a neutrophil invasion in the airways after exposure to fungal aerosols [Fogelmark *et al* 1991] which suggests that toxic pneumonitis could be caused by molds. So far, (1→3)-β-D-glucan inhalations have not produced toxic pneumonitis.

Airways inflammation is often accompanied by systemic symptoms, such as headache, fatigue and joint pains. It is likely that several of these symptoms are caused by inflammatory mediators, produced in the lung after inhalation and distributed to different parts of the body via the blood [Dunn 1992; Michel *et al* 1995].

***Mycotoxin effects*** considered to comprise memory loss, concentration problems irritability, dizziness and sleeping problems [Auger 1995]. The data suggest an effect of the toxins on the central nervous system. Some of the symptoms could, however, be caused by airways inflammation and the ensuing systemic symptoms as just described.

Persons exposed to high amounts of molds have reported loss of sensitivity, stinginess and pain in the muscles.

## Can Agents be Related to Effects?

A major difficulty relating a specific agent to a specific effect is that the exposure under field conditions is always multifactorial. Relationships found in field or epidemiological studies are never a proof of causality for a specific agent.

Many agents – known and unknown – in molds covary and furthermore the presence of mold covaries with other agents such as Gram-negative bacteria and house dust mites. In an evaluation of causative agents, information from field studies is thus never complete and information on exposure to the pure agent is needed. This information can be obtained from animal or human experiments. Obviously, the human studies are more relevant but ethical considerations impose important limitations, both for chronic exposure or exposure to certain agents such as mycotoxins. We are thus facing the scientific dilemma that, although we show relations between exposure and e. g. mycotoxin, me may never be able to obtain the scientific proof required for causality. Claims that a specific agent, either a particular microbe or a certain compound, such as mycotoxin, is the causative agent are thus uncertain. This does not exclude that a specific agent can be used as a marker for risk or as a surrogate for the important exposure.

## Conclusions

Molds contain a variety of agents with potent biological activities, either by themselves through direct contact with the cells, via metabolism or by altering the defense system against other agents.

Excessive mold exposure may cause a variety of medical effects, ranging from non-specific airways inflammation (probably the most common effect) to allergic reactions, lymphocyte related reactions such as hypersensitivity pneumonitis and cancer.

To attach any of these effects to a particular agent is difficult. There are a number of agents in mold with strong biological effects but there is very little information on causality.

While it is clear that further information on the importance of the different agents defined – and perhaps others not yet defined – requires additional research efforts, it should not be taken as an excuse, not to decrease mold exposure whenever found in higher than normal amounts and particularly when effects are present.

Thus the book of Leviticus still serves as our guideline for prevention.

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Table 1. Specific agents present in molds

| **Structural Agents** |
|---|
| Ergosterol |
| Extracellular polysaccharides |
| (1→3)-ß-D-glucan |
| **Metabolic Agents** |
| Enzymes |
| Mycotoxins |
| Volatile organic compounds |

Table 2. Diseases related to mold exposure after inhalation

| **Pulmonary Effects** |
|---|
| Airways inflammation (non-specific) |
| Hypersensitivity pneumonitis (allergic alveolitis) |
| - granulomatous pneumonitis/fibrosis |
| Decreased resistance to infectious agents |
| - haemorraghic pneumonia |
| Allergy (IgE) |
| **Systemic Effects** |
| Toxic pneumonitis |
| Central nervous system effects |
| - fatigue, headache, memory loss, irritation |
| Peripheral nerve effects |
| - loss of sensory, tactile |