

Characterization of *Aspergillus umbrosus* carbohydrate antigens by biotinylated lectins and IgG response to mannan/mannoprotein antigens in patients with farmer's lung

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Summary

Twenty-one different biotinylated lectins were used to recognize the carbohydrates of *Aspergillus umbrosus*, one of the most common microbes that patients with farmer's lung in Finland are exposed to. The glycoprotein fraction of *A. umbrosus* was bound especially well by Concanavalin A and consisted mainly of carbohydrates mannose and glucose. The carbohydrate fraction of *A. umbrosus* antigens were isolated from the crude extract of *A. umbrosus* with Con A–Sepharose affinity chromatography. Serum IgG antibodies to *A. umbrosus* mannan/mannoprotein fraction were determined in 57 patients with farmer's lung, 10 asymptomatic exposed farmers and 10 healthy controls by enzyme linked immunosorbent assay. *Candida albicans* and *Saccharomyces cerevisiae* mannan were used as controls. Patients had high levels, whereas asymptomatic exposed farmers showed moderate, and healthy controls low levels, of IgG antibodies to the *A. umbrosus* mannan/mannoprotein fraction. There were no significant differences in the mean antibody levels between the patients and controls against *C. albicans* or *S. cerevisiae* mannose fraction although in all groups more antibodies were detected against *C. albicans* than *S. cerevisiae*.

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Introduction

Farmer's lung (FL) is a form of hypersensitivity pneumonitis that results from the handling of mouldy hay or straw or other mouldy materials in the work environments of farmers. World-wide, the main causative agent of FL is *Faenia rectivirgula* (formerly *Micropolyspora faeni*) [1]. In Finland, however, patients with FL have been exposed more often to *Aspergillus umbrosus* and *Thermoactinomyces vulgaris* than to *F. rectivirgula* [2]. In fact, *A. umbrosus* is the most common microbe found in the Finnish farm environments [3]. IgG antibodies against this fungus have been found also in the sera of Danish FL patients [4]. Evidently, some antigens of *A. umbrosus* are common with *A. fumigatus* [5], but more

detailed analysis of *A. umbrosus* antigen has not been reported.

Prerequisite for the diagnosis of FL is that the farmer has been exposed to the offending antigens; otherwise the diagnosis is based on clinical findings. Internationally accepted diagnostic criteria have been presented quite recently [6]. The presence of antigen specific IgG antibodies in the patient's serum can be used as an indicator for the exposure to these antigens but the antibodies are not disease specific.

In the present study we have isolated from the crude antigen of *A. umbrosus* the carbohydrate antigens, characterized them by using biotinylated lectins, and analysed their serological significance in FL.

Materials and methods

Patient sera

Sera from 57 patients with clinically confirmed [6] FL, 10 asymptomatic farmers (exposed controls) and 10

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confirm a causative relationship between these antigens and FL. The different serological response towards *A. umbrosus* mannan, compared to *S. cerevisiae* or *C. albicans* mannans, suggests different three-dimensional structures in these fungal polysaccharides. It is known that *C. albicans* mannan is a branched α -mannose chain consisting of α -1,6-linked backbone with α -1,2-linked side chains. *S. cerevisiae* mannan is made of α -1,3-linked mannose and also β -linked mannose. The structure of *A. umbrosus* mannan is yet undetermined, but apparently differs from these.

Kurup *et al.* [12] have reported that the antigenic components of another fungus of the genus *Aspergillus*, *A. fumigatus*, might be glycoproteins, which consist of mannan, or glucose or both. Our findings are in accordance with those made by Schonheyder *et al.* [13], that the strong reactivity in ELISA is due to the presence of specially reacting glycoprotein components, rather than due to the proteins. Our immunoblotting results with sera from patients and controls against the crude antigen of *A. umbrosus* show high molecular weight diffuse carbohydrate staining seen with high absorptions in ELISA. They also revealed that the mannoprotein band of 23 kD seems to correlate with the intensity of the diffuse high molecular weight glycoprotein staining. The antigenic properties of the specific 23 kD mannoprotein of *A. umbrosus* need to be further analysed.

From the immunological point of view the three studied mannans fall clearly into three different categories: *C. albicans* mannan is apparently a classical polyclonal B-cell activator, as many other polysaccharides, inducing an IgG response in most individuals [14]. This was seen also in our study, where the IgG response to *C. albicans* mannan was elevated in all groups. *S. cerevisiae* mannan seems to be immunologically inactive and no IgG response to it was seen in any of our study groups. This is in accordance with our previous immunoblotting studies in asthmatics and healthy controls (unpublished results, Savolainen).

A. umbrosus mannan is neither immunologically inactive nor a polyclonal activator, but is likely to be a classical antigen for MHC II-restricted antibody response. Therefore it is tempting to think that the FL disease is dependent on T-cell response to a carbohydrate-containing antigen. Both CD4+ and CD8+ cells are represented in the bronchoalveolar lavage fluid of farmer's lung patients [15]. The responsiveness of these cells to the polysaccharides of *A. umbrosus* should be studied.

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