

(1→3)- β -D-glucan may contribute to pollen sensitivity

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SUMMARY

The amount of (1→3)- β -D-glucan in pollen from different plants was evaluated using the Limulus assay with a specific lysate. The amount ranged from 79 to 1800 ng/10⁶ pollen. A calculation of the inhaled dose suggests that the amount of (1→3)- β -D-glucan present during periods with a high pollen content in the air exceeds levels that cause airways inflammation.

Keywords pollen allergy inflammation (1→3)- β -D-glucan airways inflammation

INTRODUCTION

Sensitivity to pollen is a widespread phenomenon. The reactions to pollen seem to be of a dual nature. One is a rather severe bronchospasm or even anaphylactic shock and the other a more or less continuous sensation of eye irritation, swollen nose and throat irritation. The latter symptomatology is similar to that caused by inhaled irritants such as bacterial endotoxin, which causes a general inflammatory response not mediated through IgE pathways [1].

One of the many potential inflammagenic agents in nature is (1→3)- β -D-glucan, a polyglucose compound present in the cell walls of fungi, certain bacteria and plants [2,3]. It has well recognized effects on inflammatory cells, with an increase in inflammatory mediator production after acute exposure *in vitro* [4]. It alters the response to inhaled antigens [5] and augments the inflammation caused by bacterial endotoxin [6].

It has previously been demonstrated that pollen from several different plants contain (1→3)- β -D-glucan [7–9]. It protects the developing pollen during meiosis and is later destroyed by the enzyme (1→3)- β -D-glucanase to liberate the microspores. No data are available on the quantities of (1→3)- β -D-glucan present in pollen from different plant species.

In view of the above, we undertook experiments to determine the amount of (1→3)- β -D-glucan in pollen. We also analysed starch granulae from short rag weed pollen, in view of their high activity in *in vitro* models. Furthermore, we evaluated whether the amounts of (1→3)- β -D-glucan present would be sufficient to cause effects after inhalation of pollen.

MATERIALS AND METHODS

Various species of pollen were obtained from Greer Laboratories in Western Australia and Allergon (Ängelholm, Sweden). A sample of pollen was weighted and placed in a tube containing 0.3 N NaOH in saline. The tube was shaken for 10 min. The number of pollen per mg was counted in a microscope.

For the extraction of pollen starch granules, 800 mg of non-defatted rye grass pollen (*Lolium perenne*) were added to 50 ml of sterile water for irrigation (Baxter Healthcare Pty Ltd, Old Toongabbie, Australia) containing 0.05% Tween-20 and vortexed vigorously for approximately 3 min. The resulting suspension was rotated end over end for 2 h at room temperature. The suspension was then passed through a 20- μ m nylon filter (Nytal, Swiss Screens Pty Ltd, Australia) and the filtrate (with > 95% whole pollen grains removed) was collected in a 50-ml Falcon tube and centrifuged at 2800 *g* for 10 min. The resulting pellet was resuspended by vortexing in 20 ml of sterile water for irrigation and passed by syringe through a 3- μ m polycarbonate filter (Nucleopore Corp. Biosearch, Australia) cased in a Swinnex w/o filter case (Millipore, Bedford, MA). The filtrate was then centrifuged as before and the resulting pellet containing approx. 1×10^8 particles was resuspended in 1 ml sterile water and stored at 4°C in a 1.5-ml cryotube. All steps were carried out in a laminar flow hood.

To determine the amount of (1→3)- β -D-glucan, a 50- μ l aliquot of the elutriate from pollen or granules was placed in a microtitre plate together with 50 μ l of Limulus lysate specific for (1→3)- β -D-glucan (Fungitec G test; Seikagaku, Tokyo, Japan) [10]. The absorbance was read in a spectrophotometer and compared with a standard (1→3)- β -D-glucan (Pachyman; Seikagaku).

RESULTS

The results from the measurements are shown in Table 1. It is seen

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Table 1. (1→3)-β-D-glucan in pollen from different plant species

Plant	ng/mg	ng/10 ⁶ pollen
<i>Brassica napus</i> (rape)	126	1800
<i>Betula lenta</i> (birch tree)	82	910
<i>Lolium perenne</i> (rye grass)	51	570
* <i>Lolium perenne</i>	55	615
<i>Fraxinus americana</i> (ash tree)	114	1140
<i>Corylus arellana</i> (hazel)	11	79
* <i>Abrosia liatio</i> (short rag weed)	4	—
—starch granulae	115	—

*Samples from Australia.

—, Not done.

that (1→3)-β-D-glucan was found in all samples of pollen tested. The similarity between *L. perenne* from an Australian source and the same pollen from a Swedish source is remarkable. The amount of (1→3)-β-D-glucan/10⁶ pollen ranged from 79 to 1800 ng.

DISCUSSION

The determination of (1→3)-β-D-glucan was made with a biological reagent, highly specific for (1→3)-β-D-glucan [10], previously used for clinical and environmental measures [11,12]. Although the presence of (1→3)-β-D-glucan in pollen has been demonstrated previously, quantitative data have not been reported. For a risk evaluation of the amounts of (1→3)-β-D-glucan found in pollen, comparisons can be made with previous experience concerning (1→3)-β-D-glucan in mould spores and in indoor environments. In *Penicillium* and *Aspergillus*, the values are around 3.5 ng/10⁶ and in *Stachybotrys* around 130 ng/10⁶ spores [13]. (1→3)-β-D-glucan has been measured in indoor environments [14]. In one house, values up to 145 ng/m³ were found and severe asthmatic bronchitis was present in two children [15]. Increased airway responsiveness has been found at levels > 5–10 ng/m³ [16,17]. The prevalence of symptoms of nose irritation, throat irritation and cough was higher in houses with levels > 10 ng/m³ [12]. After an acute inhalation of pure (1→3)-β-D-glucan, there was an increased extent of symptoms from the nose and throat [18]. Extrapolating from these data, a dose of 0.2–0.8 × 10⁴ pollen/m³ would be sufficient to cause effects similar to the ones described above. Such levels are commonly found in outdoor air during the pollen season.

As the size of pollen is large, 10–30 μm, they will mainly deposit in the nose and the upper airways. This corresponds to the clinical experience with pollen, where the major symptoms are present in the upper respiratory tract, as opposed to mould spores, which cause symptoms in the lower respiratory tract. Based on previous data on the toxicity, it is suggested that (1→3)-β-D-glucan in pollen may cause an inflammatory effect and contribute to the development of an IgE-related sensitivity.

Research to evaluate further the role of (1→3)-β-D-glucan in

reactivity to pollen is highly warranted, as this could yield an increased understanding of a hitherto unknown causative agent and an alternative pathological mechanism for a disease common in the population, and perhaps open the pathway for prevention or at least an amelioration of the symptoms.

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