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SHORT COMMUNICATION

(1→3)-β-D-Glucan Does Not Induce Acute Inflammation After Nasal Deposition

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To assess if (1→3)-β-D-glucan, a microbial cell wall agent normally present in pollen, has the ability to produce pollenlike response, sensitive persons received a nasal deposition of two doses of (1→3)-β-D-glucan. The percentage of eosinophils and amount of eotaxin were measured in nasal lavage 30 minutes and 24 hours after challenge. No effect could be demonstrated. The absence of an inflammatory response after (1→3)-β-D-glucan application confirms earlier findings in inhalation studies.

INTRODUCTION

Microbial cell wall agents (MCWAS) play an important role in the development of nonallergic airways inflammation in occupational and general environments with microbial contamination. Gram-negative bacteria contain a lipopolysaccharide ((LPS), endotoxin) compound on their outer cell surface and dose-response relationships have been found between endotoxin in organic dusts and respiratory symptoms [1]. In fungi, the cell wall contains a particular polyglucose agent—(1→3)-β-D-glucan—that has been found to activate neutrophils in vitro with a subsequent secretion of inflammatory cytokines [2, 3]. In inhalation challenges in animals, there is an invasion of eosinophils into the airways but the classic neutrophil response is not present except if water-soluble compounds are used [4].

Apart from the fungi case, (1→3)-β-D-glucan is also present in pollen [5]. Hypothetically this agent could thus be responsible for or contribute to the inflammation in the nasal mucosa, caused by inhalation of pollen among sensitised persons. To evaluate this hypothesis experiments were undertaken where persons with a known hyperreactivity to pollen were exposed to pure (1→3)-β-D-glucan using a nasal spray. The proportion of eosinophils and amount of eotaxin were determined in nasal lavage (NAL) fluid 30 minutes and 24 hours after exposure.

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MATERIALS AND METHODS

Test persons

Test persons ($n = 11$) were recruited by advertising among university students. Inclusion criteria were known reactivity to pollen, age of 18–35 years, never smoking and without any current disease or regular medication. The study was approved by the Ethical Committee.

Glucan exposure

A purified preparation of (1→3)-β-D-glucan from *Candida albicans* was used [6]. A 5- mg aliquot was suspended in 5 mL of 0.3 N NaOH, ultrasonicated for 30 minutes, and diluted in phosphate buffered saline (PBS) till a concentration of 5000 and 500 ng/mL. Each of these doses was further diluted in PBS and 200 μL was inserted in a Biodose nasal applicator (Valois, France) that produced an aerosol of 100 μL for each of the two actuations. The resulting doses of 50 and 5 ng were chosen based upon the data on the amount of (1→3)-β-D-glucan in pollen where a typical spring exposure of 5 000 pollen/m^3 was calculated to correspond to 5 ng (1→3)-β-D-glucan/m^3 [5]. Control applications contained PBS only. The subjects were exposed in both nostrils on three occasions, at least one week apart, randomly to the high and low doses and control fluid. The exposures took place during the month of March.

Nasal lavage

The subjects underwent NAL 30 minutes prior to exposure (baseline) and 30 minutes and 24 hours afterwards. NAL was performed according to a method previously described [7]. A syringe with 6 mL PBS was connected to a nasal olive and inserted in one nostril. The fluid was slowly injected and withdrawn; this process was repeated five times. The same procedure was repeated in

the other nostril using the same fluid. The collected fluid was stored in plastic tubes in ice until centrifugation of 200 g for 10 minutes. The supernatant was removed and stored frozen at −70°C. The cell pellet was resuspended in PBS and a cell smear was prepared.

Cell counts

A cytosine cell smear preparation of the NAL fluid was stained with May-Grünewald-Giemsa and 200 cells were counted in an optical microscope at 1000× magnification, determining the proportion of eosinophils.

Eotaxin analysis

The amount of eotaxin in the NAL was analysed using an ELISA commercial preparation (Quantikine Human Eotaxin/CCL 11, R&D Systems, Abigdon, Oxon, UK) with a sensitivity of 5 pg/mL.

RESULTS

A very large proportion of eosinophils was found among two subjects in the control tests (80% and 25.5%) as compared to the average of the group which was 1.6. None of these persons showed an increase in the proportion of eosinophils after application of (1→3)-β-D-glucan. Table 1 shows the proportion of eosinophils among cells in the NAL fluid of the different groups, excluding the persons with high initial values. No differences were seen between (1→3)-β-D-glucan exposures and control exposures.

Table 1. Eosinophils in nasal lavage (percentage of total cells counted). Mean values and ranges in parentheses (excluding 2 persons with high baseline values, see the text).

Exposure	Before	30 min	24 h
Control exposure	1.65 (0–9.5)	1.1 (0–4.0)	3.2 (0–24)
Glucan (low dose)	0.7 (0–4.0)	0.5 (0–2.3)	0.8 (0–3.3)
Glucan (high dose)	0.8 (0–2.0)	0.9 (0–3.4)	1.6 (0–9.1)

Figure 1 shows the amount of eotaxin in the NAL fluid. The distribution of values was the same, irrespectively of the instillation agent.

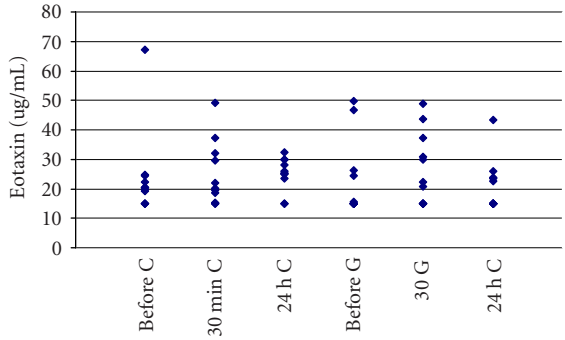

Figure 1. The amount of eotaxin in nasal lavage after the application of (1→3)-β-D-glucan (G) or PBS (C).

DISCUSSION

The present study is of an exploratory nature and the number of subjects small. The dose in the nose was calculated basing the exposure on a typical spring exposure of 5 000 pollen/m^3 which means that the doses of (1→3)-β-D-glucan used were well in the range of the normal environmental dose [5].

The absence of an inflammatory reaction after exposure to (1→3)-β-D-glucan agrees with results from inhalation studies in animals [8, 9, 10]. On the other hand, a marked nasal swelling and increased amounts of interleukin-8 were found in a study where subjects inhaled floor dust, spiked with (1→3)-β-D-glucan (Kjaergaard, personal communication). Whether this difference reflects the presence of particles in the floor dust or a higher dose level is not clear.

In summary, the results do not support the hypothesis that (1→3)-β-D-glucan induces the inflammatory response seen after exposure to pollen in sensitised subjects.

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