

Potential of Histamine Release by Microfungal (1→3)- and (1→6)-β-D-Glucans

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Abstract: (1→3)-β-D-Glucans, a cell wall component in most microfungi, are suggested to play a role in the development of respiratory and general symptoms in organic dust-related diseases. The mechanisms by which they induce these effects are, however, not clear. In the present study, mediator release and its potentiation by the (1→3)-β-D-glucan as well as by the (1→6)-β-D-glucan found in yeast and other fungi were therefore examined. Blood leucocytes from healthy volunteers and from patients allergic to house dust mite were incubated with (1→3)-β-D-glucans with increasing 1,6-branchings: curdlan [a linear (1→3)-β-D-glucan], laminarin and scleroglucan, and furthermore with pustulan, a linear (1→6)-β-D-glucan. Histamine release was not observed on exposure to the glucans only, but in the presence of anti-immunoglobulin E (IgE) antibody or specific antigens, all the glucans investigated led to an enhancement of the IgE-mediated histamine release. The glucans induced a significant potentiation of the mediator release when present at concentrations in the range of $2\text{--}5 \times 10^{-5}$ M. These results suggest that (1→3)-β-D-glucan as well as (1→6)-β-D-glucan aggravates IgE-mediated histamine release. Knowledge concerning the effects of glucans on immune responses may be of importance for understanding and treating inflammatory and allergic diseases.

(1→3)-β-D-Glucan is a cell wall component found in most microfungi. In damp and mouldy buildings, the glucan has been used as a putative marker of microfungal growth in the examination of indoor mould exposure as a risk factor for asthma and other adverse health effects [1–4]. A relation has been found between the indoor level of (1→3)-β-D-glucan and the proportion of persons reporting symptoms indicative of airway inflammation [1,2]. Upper airway inflammation, verified by nasal lavage, has also been found in waste and compost workers exposed to bioaerosols containing (1→3)-β-D-glucan, endotoxins and fungal spores, indicating a possible role of these agents in the inflammatory response [5,6]. The ability of (1→3)-β-D-glucan to induce histamine release has not been investigated, but the production of cytokines upon exposure to (1→3)-β-D-glucans has been examined *in vitro*. The results obtained indicate that (1→3)-β-D-glucans induce the synthesis of IL-1, IL-6 and tumour necrosis factor-α in murine macrophages and human blood cells, and additionally human blood cells show increased production of IL-8 when exposed to (1→3)-β-D-glucans [7–11]. However, linear (1→3)-β-D-glucan with a linear (1→6)-β-D-glucan moiety also induce enhanced IL-6 synthesis and enhanced lipopolysaccharide-induced tumour necrosis factor-α production [12]. Because both (1→3)-β-D-glucan and (1→6)-β-D-glucan are found in fungi and yeast, it is

important to clarify whether (1→6)-β-D-glucans also have the capacity to induce release of inflammatory mediators. Therefore, the present study included both glucans in the study of histamine release, a mediator in allergic, immunological and inflammatory diseases.

Materials and Methods

Participants. Ten healthy volunteers and three voluntary patients allergic to house dust mite (age 28–56 years) were included in the study. The study was carried out in accordance with the Declaration of Helsinki and Guidelines on Good Clinical Practice.

Materials. Anti-immunoglobulin E (IgE) antibody preparation (solution of monoclonal anti-human IgE 1 mg/ml, conserved in glycerol, samples kept at –20°C) was a kind gift from Novozymes A/S, Denmark. House dust mite allergen extract (D. Farinae, 10 HEP, Soluprick SQ) was obtained from ALK-Abelló, Denmark. The histamine release was measured after exposure to the following glucan samples: linear (1→3)-β-D-glucans included curdlan showing no branching (synonym: paramylon, Sigma-Aldrich, Fluka 89862, St. Louis, MO, USA), laminarin containing some branchings of (1→6)-β-D-glucans (Sigma-Aldrich L9634) and scleroglucan with approximately 30% branchings of (1→6)-β-D-glucans (Actigum CS11 provided by Sanofi Bioindustries, with reduced molecular weight according to Sletmoen and Stokke [13]. Furthermore, a linear (1→6)-β-D-glucan pustulan (Calbiochem 540501) was included.

Histamine release from blood leucocytes. Blood was drawn by venipuncture, anticoagulated with ethylenediaminetetraacetic acid at a final concentration of 0.01 M, and mixed with the 2-fold volume of a 0.9% NaCl solution. Leucocytes with approximately 2% basophils were isolated from the blood samples by the Percoll gradient method (density 1.080 g/ml), washed and suspended in glucose-free Tris-AMC [14].

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The amount of glucan-induced histamine release was determined in samples of 50 μ l cell suspension mixed with 10 μ l of glucan dissolved or suspended in Tris-AMC and incubated for 40 min. at 37°C. In order to examine whether the presence of the glucans had an enhancing effect on the histamine release induced by anti-IgE antibodies or specific antigens, the cell suspension was incubated with a combination of glucans and either anti-IgE antibody or allergens. The intracellular content of histamine in the sample prior to the experiment and the spontaneous histamine release during the experiment was examined in the absence of these agents and by using no incubation and incubation, respectively. After centrifugation (2000 $\times$ g, 10 min.), the supernatant, in addition to released histamine, also contains proteins and other substances that may interfere with the histamine analysis. Therefore, the supernatant was discarded and the residual histamine in the cell sediment was determined, essentially according to Stahl Skov and Norn [14]. The residual intracellular histamine was released by adding 40 μ l 0.59% HClO₄ to the cell sediment. Thereafter, a mixture of 0.1 mg *o*-phthalaldehyde dissolved in 50 μ l methanol and 450 μ l 0.15 M NaOH was added. After 4 min., 500 μ l 0.53 N H₃PO₄ was added and the amount of fluorescence light emitted by the solution was determined at 355/455 nm (excitation/emission). The release of histamine was expressed as a percentage of the total histamine content of the sample. All values were corrected for the spontaneous release, which amounted to up to 5%.

Statistics. A statistical analysis was performed using the Wilcoxon rank-sum test. P-values of <0.01 were considered significant.

Results

Examination of glucan-induced histamine release.

The capability of the glucans to trigger basophil histamine release was examined in blood leucocyte suspensions obtained from the 13 people included in the study. The glucan, either curdlan, laminarin, scleroglucan or pustulan, was examined in final concentrations ranging from 10⁻⁶ to 10⁻¹ mg/ml leucocyte suspension. The amount of histamine release determined on exposure of the blood leucocytes to the different polysaccharides did not exceed the spontaneous histamine release ($P < 0.01$).

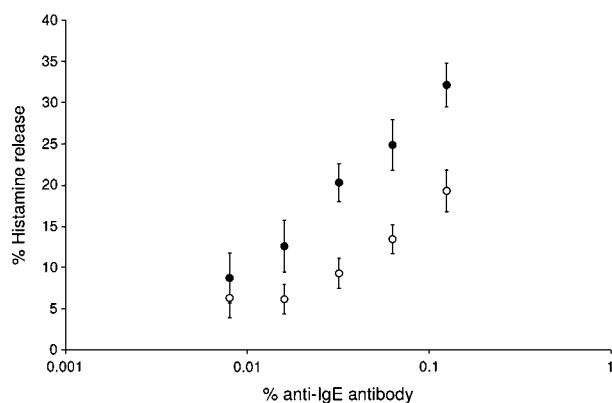


Fig. 1. Potentiation by curdlan of immunoglobulin E (IgE)-mediated histamine release in suspensions of blood leucocytes from eight healthy volunteers. The results shown are obtained for cells incubated with anti-IgE antibody (open circles) and in combination with 10⁻¹ mg/ml curdlan (closed circles). X-axis: % anti-IgE antibody preparation. Mean $\pm$ S.E.M.; $P < 0.01$ for the combination versus anti-IgE antibody in all concentrations except for the lowest two concentrations.

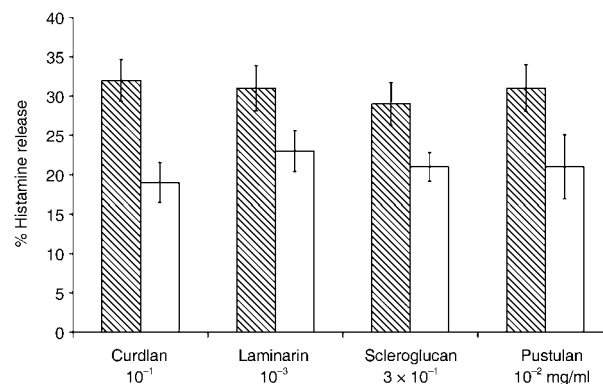


Fig. 2. Potentiation by glucans of immunoglobulin E (IgE)-mediated histamine release in suspensions of blood leucocytes from seven to nine healthy volunteers. The figure shows the lowest concentration of the glucans causing a significant potentiation. Cells incubated with anti-IgE antibody (open column) and in combination with the glucan (hatched column). Mean $\pm$ S.E.M.; $P < 0.01$ for the combination versus anti-IgE antibody.

Potentiation of histamine release by glucans.

A potential potentiating effect of the glucans on anti-IgE antibody-mediated histamine release was examined in leucocyte suspensions obtained from 7–9 of the healthy volunteers. The cell samples were incubated with increasing concentrations of anti-IgE antibody to obtain a histamine release between 10% and 20%. In the presence of curdlan, examined in final concentrations from 10⁻⁶ to 10⁻¹ mg/ml leucocyte suspension, a significant increase in the histamine release was observed for curdlan in the concentration of 10⁻¹ mg/ml ($P < 0.01$). Figure 1 shows the potentiation. Similar potentiation curves were obtained for the other glucans. A significant enhancement of histamine release was obtained on addition of laminarin in concentrations from 10⁻³ to 10⁻¹ mg/ml, scleroglucan in the concentration 3 $\times$ 10⁻¹ mg/ml and pustulan in concentrations from 10⁻² to 10⁻¹ mg/ml. Column diagrams, depicting the lowest concentrations of the glucans causing a significant potentiation, are presented in fig. 2. The threshold level corresponded to 2–5 $\times$ 10⁻⁵ M glucan, regardless of the glucan structure [i.e. (1 $\rightarrow$ 3) or (1 $\rightarrow$ 6) linkages] (table 1). A glucan-induced potentiation of IgE-mediated histamine release was also observed in three participants allergic to house dust mite when cells were challenged with *D. Farinae* in the presence of 10⁻² mg/ml

Table 1.

Data of potentiation.

	Linear	Branchings	Potentiation*	
			(mg/ml)	MW
Curdlan	β -1,3		10 ⁻¹	500.000
Laminarin	β -1,3	some β -1,6	10 ⁻³	5.000
Scleroglucan	β -1,3	30% β -1,6	3 $\times$ 10 ⁻¹	540.000
Pustulan	β -1,6		10 ⁻²	20.000

*Potentiation, lowest dose, corresponding to 2–5 $\times$ 10⁻⁵ molar concentration. MW, molecular weight.

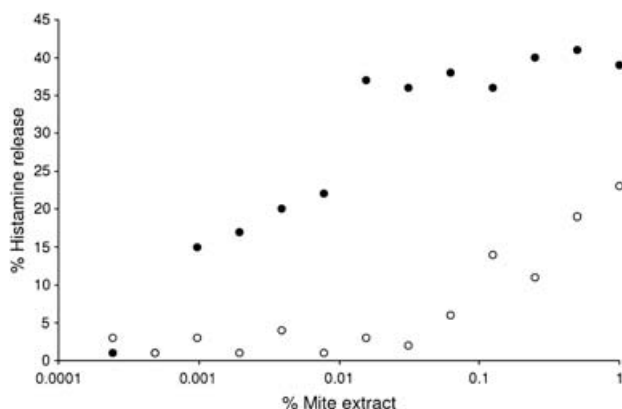


Fig. 3. Potentiation by laminarin of mite allergen (*D. Farinae*) induced histamine release in cells from a subject allergic to house dust mite. Cells incubated with *D. Farinae* (open circles) and in combination with 10^{-2} mg/ml laminarin (closed circles).

laminarin. Laminarin caused a shift of the dose-response curve to the left (i.e. to smaller concentrations of the allergen), causing an equal release of histamine (fig. 3). A shift of one, two and three decades was observed by laminarin in these persons.

Discussion

The present *in vitro* study of histamine release from human blood leucocytes showed that neither (1→3)- β -D-glucan nor (1→6)- β -D-glucan caused histamine release from the basophils. The potentiating effect of relatively pure glucan samples on histamine release has not previously been studied. Results obtained in investigations of the potentiating effect of dextran [(1→3)-branched (1→6)- α -glucan] and zymosan [a source of (1→3)- β -glucans, mannan and other substances] are available. In certain previous studies, rat peritoneal mast cells have been found to respond directly to dextran [15]. The results obtained in the present study did not indicate a direct interaction of glucan with human basophils leading to histamine release. These results are thus in accordance with previous results obtained for human cells, because anaphylactoid or complement-mediated reactions are involved in dextran- and zymosan-mediated histamine release [16,17]. However, the present investigation revealed a potentiating effect of both (1→3)- β -D-glucan and (1→6)- β -D-glucan on histamine release induced by anti-IgE antibody as well as by specific antigen in patients allergic to house dust mite. Enhanced mediator release has previously been observed on addition of endotoxins [18,19]. It is unknown whether the glucan preparations used in the present study contain impurities of endotoxins. However, the threshold of potentiation was equal for all the glucans investigated, because the lowest concentration of glucan causing a significant enhancement of histamine release was equal to $2-5 \times 10^{-5}$ M. It is interesting to note that this was also the case regardless of the 1,3- or 1,6-structure. The suggested augmentation of antigen-stimulated histamine release is supported by similar experiments with zymosan [20].

It can be speculated whether an enhancement of IgE-mediated release of histamine by the glucans may be a potential factor in the development or aggravation of respiratory symptoms and asthma by the exposure to moulds in damp and mouldy buildings and in the handling of waste and compost. Previous studies have focused on the role of fungi, bacteria and endotoxins, and an association between these agents in dust or air and respiratory and general symptoms has been found [21–23]. Glucans with β -(1→3)- and β -(1→6)-glucosidic linkages are found in fungi and yeast as well as in some bacteria [8,24–27]. The (1→3)- β -D-glucan is the only compound that has been used as a marker of fungal level in air and dust, and a relation has been observed between the indoor glucan level and the proportion of people reporting symptoms indicative of airway inflammation [1,2]. Only small effects have been determined in exposure assessments with short-term exposure to inhaled (1→3)- β -D-glucan. It is, however, not clear how well such assessments represent long-term airborne exposures [28,29]. Multiple factors and their interactions, including immunological as well as non-immunological mechanisms, may be involved in the cascade of events leading to adverse health effects in organic dust-related diseases [30,31], and glucans might be one of these factors. Another interesting aspect is that pollen from birch and other plant species contains (1→3)- β -D-glucans that might contribute to pollen sensitivity [32].

In animal studies, oral administration of fungal β -D-glucan have been shown to produce an antitumour effect, and in patients with colorectal cancer and gastric cancer, it improves remission and survival rate when used as an adjunct to surgery and standard chemotherapy [33–36]. The mechanism leading to the antitumour effect of β -D-glucan may include activation of cytotoxic T cells, natural killer cells or phagocytic cells by priming of their iC3b receptors [33,37,38]. It is still unclear whether enhanced histamine release induced by β -D-glucan also contribute to the antitumour effect, because studies have shown that the effect of IL-2 is improved by including histamine in the immunotherapy [39,40].

Our results reveal that (1→3)- β -D-glucan and (1→6)- β -D-glucan enhance the release of histamine from human blood cells. These results suggest a modulating role of these glucans on the immune response in the sense that they may lead to development or aggravation of inflammatory and allergic diseases and to improvement of immunochemotherapy.

Acknowledgements

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