AN INVESTIGATION INTO THE EFFECT OF LIPOPOLYSACCHARIDE AND (1→3)-β-D-GLUCAN EITHER IN COMBINATION OR SEPARATELY ON THE DEGRANULATION OF MAST CELLS ISOLATED FROM THE HUMAN LUNG

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Abstract

Dusts containing microbial agents have been implicated in a range of respiratory disorders such as asthma, extrinsic allergic alveolitis and organic toxic dust syndrome. The lung inflammation shown in these diseases is often linked to the mast cell., which is of central importance to the immediate and chronic effects of these conditions as indicated by bronchial hyperreactivity, through the release of inflammatory mediators such as histamine, prostaglandins and leukotrienes.

This work examines the histamine releasing effect of Lipopolysaccharide and (1→3)-β-D-glucan, both of which are found in cotton and other organic dusts, on the degranulation of mast cells isolated from the human lung.

Introduction

A number of key studies have identified Endotoxin, also referred to as Lipopolysaccharide (LPS) and derived from gram negative bacteria, as an agent capable of causing important physiological changes in biological systems and thus being of importance to the pathology of a range of organic dust related diseases. More recently (1→3)-β-D-glucans derived from the inner cell wall of various yeasts and plant material and consisting of glucopyransoyl subunits connected by (1→3)-β polyglucoside linkages have been similarly implicated in the disease process of certain pulmonary disorders. Both these agents are found in areas of organic dust contamination, in particular areas subject to high levels of cotton and grain dust, and are therefore linked to a range of dust related respiratory conditions (Rylander and Jacobs 1994, Fogelmark et al 1994).

An important cell in the progression of such respiratory problems is the mast cell which is known to play a central role in airway hypperresponsiveness, and as recent evidence suggests in chronic inflammatory processes. Mast cells are known to be activated immunologically via cross-linking of surface bound IgE or by a variety of non-immunological agents. These cells produce a wide range of mediators including biogenic amines, heparin, proteases, prostaglandins, leukotrienes, kinins and cytokines (Metcalfe et al 1997). They are found in the trachea, alveolar septa, bronchi and bronchioles of the lung being located beneath the epithelium. The mast cells of the lung belong to a particular group of referred to as tryptase containing type (MC_T) found in the mucosa. Owing to its location and varied range of mediators produced the mast cell is not only ideally situated for producing the acute phases of respiratory distress but may also be of vital importance to orchestrating the more chronic conditions of various disorders of the pulmonary system.

Bacterial and viral products have been shown to modulate or activate the release of mediators from the mast cell (Lutton et al 1995, Larsen et al 1996), this work therefore examines the interaction of the mast cell isolated from human lung tissue with (1→3)-β-D-glucan derived from Bakers Yeast and Lipolysaccharide from E-coli by analysing cell activation through the release of histamine. The pathophysiology of organic dust related diseases is poorly understood and investigation of such interactions with dust and mould contaminants should provide valuable information as to the cellular mechanisms of a range of dust related respiratory diseases.

Materials and Methods

Tissue

Macroscopically normal Human lung tissue was kindly donated by the University of Wales College of Medicine, Pathology Department.

Materials

All materials unless otherwise stated were purchased from the Sigma Chemical Company and diluted in HEPES-tyrodes buffer. The histamine analysis plates were obtained from Referencelaboratoriet Copenhagen, Denmark.

Methods

Mast Cell Isolation (Ali and Pearce 1985)

The human lung tissue was chopped into $1mm^3$ pieces and placed in full Hepes buffer containing N-2_Hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES) 10mM, $CaCl_2.5H_2O$ 1mM, NaH_2PO_4 0.4mM, NaCl 137mM, glucose 5.6mM. Collagenase was added at 20IU/ml with BSA at 1mg/ml and incubated with mixing at 37°C for 90 minutes. After which the mixture was filtered through Nylon gauze (Fisher) pore size 150µm. The cell mixture was centrifuged at 200g for 5 minutes at 4°C. The cells were then centrifuged similarly and resuspended in warm buffer for use in experiments.

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Mast cell numbers were assessed by alcian blue staining (Gilbert and Ornstein 1965) or cytospin stained with Leishmanns stain - typically purity was 3-5% and viability in excess of 90% as assessed by trypan blue staining.

Agent Incubation

These experiments were carried out using 150µl of the various cell isolates to which 30µl of the agent to be examined and 150µl buffer were added. In the case of calcium free experiments the calcium was removed from the incubation buffer and 1mM EDTA added to ensure removal of all intrinsic calcium. The cells were incubated in polypropylene tubes at 37°C for 15 minutes. The reaction was stopped by addition of excess cool buffer thereby diluting the mixture. The mixture was immediately centrifuged at 200g for 5 minutes and the supernatant poured off. The cell pellet was resuspended in an equivalent amount of buffer. Finally the supernatant and cell pellet tubes were frozen to disrupt the cells prior to assaying for histamine content.

Histamine Fluorometric Assay (Shore st al 1959)

The histamine assay was carried out using a novel method applying glass microfibre attached to the bottom of Elisa plates too which histamine binds (Skov et al 1984). The samples were incubated in each well for one hour at 37°C prior washing to remove unbound molecules and then dried overnight. The final part of the procedure post-preparation was carried out by the Referencelaboratoriet in Denmark under the supervision of Prof P. Stahl Skov, this involved spectrophotometrically measuring the o-phthaldialdehyde bound histamine released from the glass fibres, samples of standard were used with the plates to determine histamine levels in ng/ml.

Results

Histamine release was determined by calculating the percentage histamine in the supernatant based on the total in the cell pellet and supernatant histamine after correction for spontaneous release. Spontaneous release for the results shown was typically between 10-15%.

Results shown as means ± SEM for the number of observations stipulated (* denotes significance at $p > 0.05$ unless otherwise stated calculated by the students t-test).

The Human Lung mast cells show a definite increase occurred on the addition of LPS particularly at 0.01mg/ml, where as Bakers Yeast (BY) glucan showed a more uniform dose-response distribution across the concentrations used (see figures 1 and 2).

It can be seen that when applied in combination BY glucan/LPS show no significant difference with the cell activation observed when the agents are added separately. However although there is no change when compared to the sole agents there is also no additive effect suggesting that one of the agents is solely responsible for the cell activation. On comparing these results it appears that it is most likely that glucan is responsible for the histamine release when combined with LPS and it in turn inhibits or nullifies any effect that LPS may have on cell activation(see figures 3-5).

There appears to be a significant relationship between the absence of calcium ions and the histamine releasing effects of glucan (Figure 6). Unusually the presence of calcium appears to be inhibiting the releasing effects of BY glucan especially at the lower concentrations. A similar result has been observed with rat peritoneal cell mast cells suggesting that the effect is not cell-type specific. However as with all of these results it is important to note that the cell system used was not pure and that interaction with other cells may well be the cause of the observed phenomenon.

Discussion

It appears that unlike that observed with the guinea-pig lung mast cells glucan and LPS do not increase the histamine release relative to the agents alone (Gregory and Nicholls 1999). This suggests that these agents do not interact cooperatively and are therefore possibly active through different mechanisms. It also seems probable that glucan is the main agent of activation and when added together may well bind LPS thereby negating any activating influence that agent may have (see results).

However, BY glucan did significantly release histamine and therefore activate mast cells up to 28% of the total available for release. However it is found that this is highly variable and this may be due to the interaction of other cells in the system or the result of the particular glucan used.

Although these levels of glucan and LPS are much greater than those experienced in most areas of high microbial contamination they do suggest that glucan at particularly high concentrations and in combination with LPS at lower concentrations may exacerbate the inflammatory response of the lungs by low-level release of mediators over a period of time. This effect is most probably multifactorial interacting with a range of other fungal and bacterial contaminants found in dusty environments. Furthermore other cells in tissue derived cell isolate may influence the activation of the mast cells in particular macrophages and eosinophils which are known to be activated by such agents.

Of particular interest is the observation that the removal of calcium ions from the extracellular medium rather than blocking the release of histamine by glucan potentiates the effect resulting in a highly significant release at low

concentrations, not observed in the presence of calcium. The initial release of histamine is dependent on activation of intracellular stores of calcium which is usually potentiated by the influx of calcium from the external environment. It may be that the glucan adopts a different conformation in the absence of calcium and this in turn causes the change in the histamine releasing effect of this particular agent with respect to the internal stores. Again variations in the effectiveness of the gluon used from Sigma may account for this effect as may further interaction with other cells in the system. Also it has been found by other workers that a glycoprotein identified on the rat mucosal type mast cell line RBL-2H3, known as the mast cell function-associated antigen, which is similar in structure to the calcium dependent animal lectins (C-type lectins) is able to bind various carbohydrates and inhibit the antigenic release of histamine. It is possible that glucan binds this receptor thereby inhibiting histamine release. Thus removal of calcium would explain the increase in observed histamine release through activation of the internal calcium stores (Binsack and Pecht 1997). However, it should be noted that there may also be physical disruption of the mast cells on binding of the glucan particles thereby causing the disruption of the cell membrane and release of the stored histamine, analysis of other mediators that are not preformed is currently ongoing in an attempt to identify the possible mechanism of action.

The high variability found within the experiments suggests that only certain samples were susceptible to glucan. This observation is similar to the differences noted between people of the same household who often have varying responses to the indoor air of the home, with some members of the same family showing breathing problems while others have no such symptoms (Thorn and Rylander 1998). The observed activation of the mast cell, through the release of histamine, by addition of glucan and LPS will have important consequences for the concurrently observed eosinophilia associated with glucan and neutrophilia that occurs with LPS (Fogelmark et al 1994), suggesting a role for the release of cytokines in particular TNF-α, IL-6 and IL-8 possibly by the mast cell.

In conclusion this information further establishes a possible link between glucan/LPS and mast cell activation, showing the effect to be variable and highly complex. As a result of the diverse range of mediators that can be released by the mast cell, in particular various cytokines, this will in turn influence acute and chronic inflammation both immunological and non-immunological, and in turn the respiratory and mucosal disorders caused by exposure to air containing high levels of these contaminants.

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Results

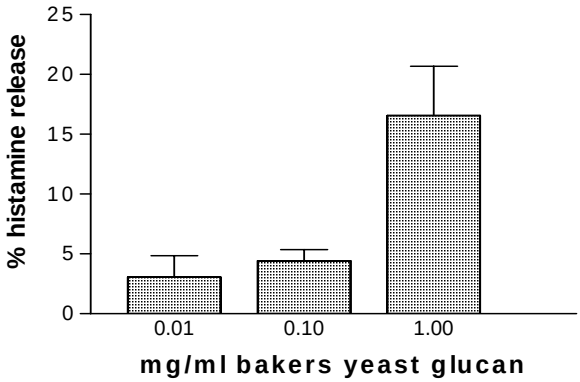

Figure 1. Percentage histamine release from human lung mast cells incubated for 15 minutes with bakers yeast glucan (n = 3 - 5 ± SEM).

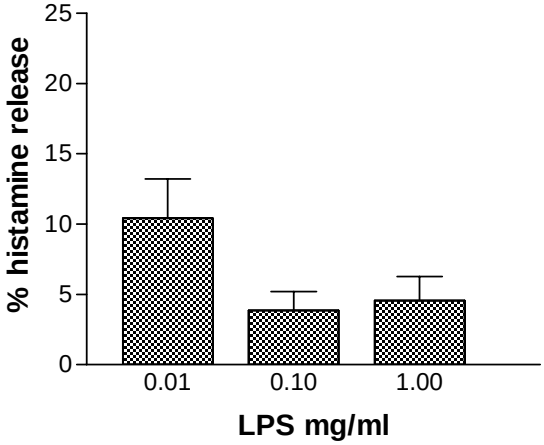

Figure 2. Percentage histamine release from human lung mast cells incubated for 15 minutes with LPS (n = 3 - 5 ± SEM).

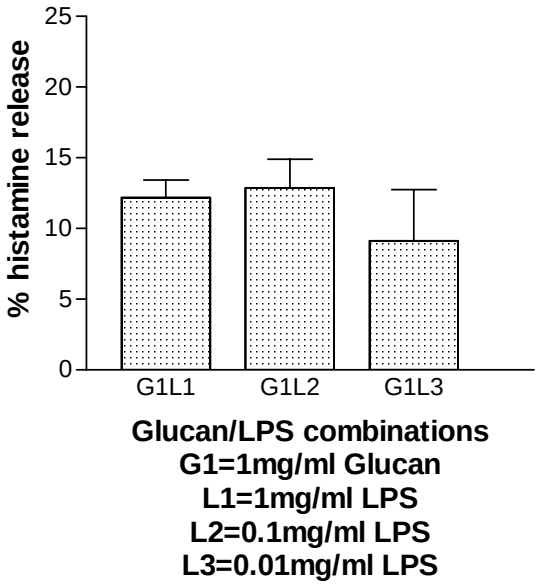

Figure 3. Percentage histamine release from human ling mast cells incubated for 15 minutes with Glucan at 1 mg/ml and a range of LPS concentrations (n = 3 - 5 ± SEM).

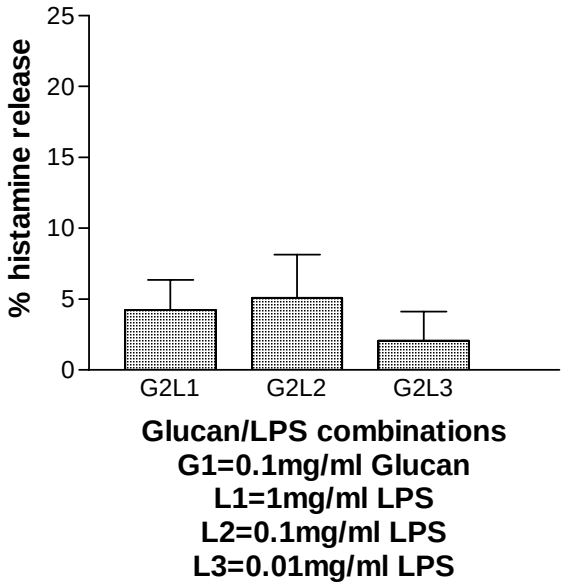

Figure 4. Percentage histamine release from human lung mast cells incubated for 15 minutes with Glucan at 0.1 mg/ml and a range of LPS concentrations (n = 3 - 5 ± SEM).

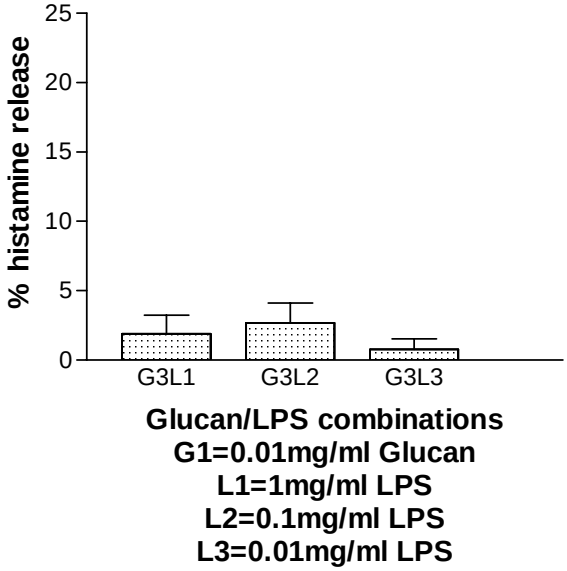

Figure 5. Percentage histamine release from human lung mast cells incubated for 15 minutes with Glucan at 0.01 mg/ml and a range of LPS concentrations (n = 3 - 5 ± SEM).

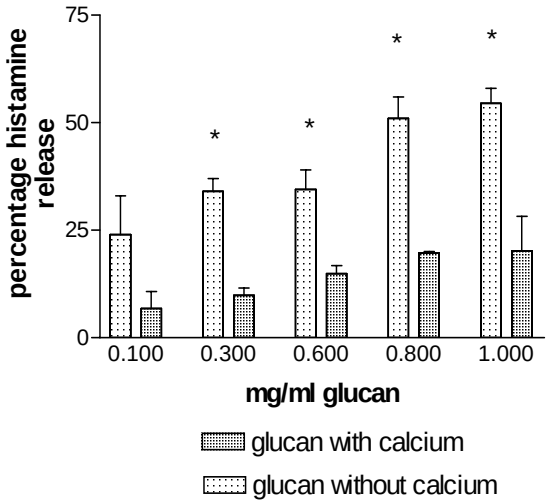

Figure 6. Human Lung mast cells incubated with Glucan with/without Calcium (n = 3 ± SEM).