

## Effect of lipopolysaccharide (LPS) and (1→3)- $\beta$ -D glucan either alone or in combination on rat peritoneal mast cells: a preliminary study

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Mast cells are known to play a central role in a range of inflammatory disorders including airway hyperresponsivity. Their role is only now becoming more fully understood; not only do they produce immediate effects, but they also help to coordinate chronic effects through the release of cytokines.

Previous work carried out by this group examined the effect of the organic dust components LPS (endotoxin), a component of Gram-negative bacterial cell membranes, and (1→3)- $\beta$ -D-glucan a fungal cell wall component, on mast cells derived from guinea pig lung tissue Gregory and Nicholls (1997). The present study aims to examine the effects of LPS and (1→3)- $\beta$ -D-glucan either alone or in combination, on mast cell enriched cell isolates or purified mast cells obtained from the rat peritoneum, measuring histamine release as a marker of activation.

Mast cells show a high degree of heterogeneity, the peritoneal mast cells show notable differences to those obtained from lung tissue, and provide a useful comparison to examine the effects of these dust components.

Peritoneal mast cells from Sprague-Dawley male rats (200-400g) were obtained by lavage. Purified mast cells were obtained by a 30/80% discontinuous density gradient centrifugation Marshall et al. (1996) giving a greater than 90% mast cell population. Soluble Glucan and LPS, from Sigma, were incubated for 15 mins at 37°C with the cells. Compound 48/80 was employed as a positive control. Levels of histamine in both the cell pellet and supernatant were then examined using a modified OPT fluorescence assay based on the method of Shore et al (1959). Spontaneous release was typically less than 10%.

**Table 1:** Histamine release from rat peritoneal mast cells purified and unpurified - preliminary results (n=3, values  $\pm$  SEM)

Stimulus	Histamine Release % purified	Histamine Release % unpurified
Compound 48/80 1 $\mu$ g/ml	75.9 $\pm$ 9.1	85.2 $\pm$ 8.9
Glucan 100 $\mu$ g/ml	1.5 $\pm$ 0.4	0.7 $\pm$ 0.6
LPS 1 $\mu$ g/ml	1.2 $\pm$ 0.7	2.3 $\pm$ 1.9
Glucan 100 $\mu$ g/ml + LPS 1 $\mu$ g/ml	21.6 $\pm$ 12.0	3.4 $\pm$ 1.2

The results above show only those obtained from animals who responded to the combined treatments, typically this was about 1 in 10, suggesting an animal specific effect. From Table 1 it can be seen that Glucan (100 $\mu$ g/ml) and LPS (1 $\mu$ g/ml) singly were ineffective.

However, in combination there was a significant release of histamine from purified mast cells. This may reflect an important synergy relevant to a range of inflammatory conditions including occupational asthma arising from inhaled organic dusts.

P.J.N. is in receipt of a grant from the British Cotton Growing Association Ltd.

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