Human Lung Microvascular Endothelial Cells Release CXCL8 In Response To The NOD1 Agonist iE-DAP And The TLR4 Agonist LPS

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Inflammation in blood vessels is associated with vascular dysfunction in sepsis and with chronic atheromatous disease. It is important to understand how bacteria interact with the vasculature in order to develop new pharmacological targets for cardiovascular disease.

We have previously shown that stimulation of the pattern recognition receptor NOD1 (which recognises peptidoglycan predominantly from gram negative bacteria) induces NOSII in rodent vascular smooth muscle causing vascular dysfunction in vitro and septic shock in vivo. However the response of human vascular smooth muscle to NOD1 stimulation is relatively weak unless co-stimulated with TLR4. The aim of the current study was to understand the role of NOD1 and TLR4 in human endothelial cells.

Briefly Human Lung Microvascular Endothelial Cells (HLMVEC) derived from healthy donors were cultured in 96-well plates in Endothelial Cell Basal Medium-2 (Clonetics®) supplemented with foetal calf serum and appropriate endothelial growth factors. Hydrocortisone had been removed from the cell culture medium 48 hours prior to plating. Cells were either untreated or treated with the NOD1 agonist iE-DAP (0.01-10μg/ml) and the TLR4 agonist LPS (0.01-10μg/ml) for 24 hours (n=15; using cells from 3 separate donors). Cell activation was measured by the release of CXCL8 using ELISA.

LPS induced concentration dependent CXCL8 release at 0.01μg/ml and above whilst iE-DAP induced concentration dependent CXCL8 release at 1μg/ml and above (Figure 1). These data show that stimulation of NOD1 induces inflammatory signalling independent of TLR4, strengthening our hypothesis that NOD1 is an important target in the vasculature.

Figure 1: Release of CXCL8 from HLMVEC stimulated with LPS or iE-DAP. Data is mean±SEM for n=15 using cells from 3 separate donors. Responses were compared to respective controls using one-way ANOVA followed by Dunnett's post-test. A p value <0.05 was taken as significant and is represented by *.

* p<0.05, 1 way ANOVA with Dunnett's post-test.