

the VAP and non-VAP groups (Abstract S77 Figure 1). Soluble TREM-1 levels were not significantly different between the groups. There was no difference in peripheral blood white cell count, CRP, expression of CD11b, L-selectin and inflammatory cytokines between VAP and disease controls.

**Conclusion** The BALF/blood ratio of monocytic surface TREM-1 discriminates between the VAP and non-VAP groups. Measurement of surface TREM-1 using a two-compartment index may have utility in diagnosing VAP.

## REFERENCES

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## S78 DETERMINANTS OF MACROPHAGE RESPONSES TO *STREPTOCOCCUS PNEUMONIAE*

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**Introduction and Objectives** *Streptococcus pneumoniae* is the commonest cause of pneumonia and associated with marked inflammatory responses that underpin its immunopathogenesis. Surprisingly little is known about the molecular determinants of host–pathogen interactions that mediate these responses. We have studied the role of the pneumococcal capsule and surface lipoproteins in innate immune responses by macrophages that comprise the first line of cellular immune defence within the lung.

**Methods** Human macrophage responses to wild type *S pneumoniae* (TIGR4) and isogenic mutant strains deficient in capsule (P1672) or surface lipoproteins ( $\Delta$ lgt) were investigated at the level of intracellular signalling, genome-wide transcriptional profiling and at protein level by cytokine ELISA.

**Results** Unencapsulated bacteria invoked greater activation of the classical NF $\kappa$ B pathway, suggesting that the capsule may serve to inhibit some innate immune host pathogen interactions. In contrast, the  $\Delta$ lgt strain showed attenuated activation of NF $\kappa$ B, suggesting that lipoproteins are important ligands for innate immune recognition of pneumococci. Transcriptional responses to both unencapsulated and  $\Delta$ lgt strains showed marked differences to wild type pneumococci. However, quantitatively, major gene expression changes were preserved in the mutant strains. Despite the divergent effects on NF $\kappa$ B activation, both unencapsulated and  $\Delta$ lgt strains showed attenuated responses amongst these genes, although some key responses such as upregulation of TNF $\alpha$  were equivalent in all strains. Transcription factor enrichment analysis was conducted for the list of genes up-regulated by each strain to obtain new insight into the different pathways by which pneumococci may activate inflammatory responses. As expected, genes up-regulated by the wild type strain were enriched only for the NF $\kappa$ B family. In keeping with the signalling data, the  $\Delta$ lgt strain was not enriched for NF $\kappa$ B but only the PPAR $\gamma$ -RXR transactivator, and the unencapsulated strain was highly enriched for NF $\kappa$ B and a raft of other transcription factors.

**Conclusion** Pneumococcal capsule and lipoproteins are important determinants of inflammatory responses to pneumococci. Our data suggest that the capsule inhibits multiple innate immune signalling pathways and that lipoproteins are critically important for activation of the canonical NF $\kappa$ B pathway. TNF $\alpha$  responses are independent of capsule and lipoproteins.

## S79 POTENTIAL DIAGNOSTIC SIGNIFICANCE OF NEUTROPHIL PROTEASES IN VENTILATOR-ASSOCIATED PNEUMONIA

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**Introduction and Objectives** The clinical diagnosis of ventilator-associated pneumonia (VAP) remains notoriously difficult, as several non-infective conditions mimic VAP. Microbiological confirmation of the diagnosis using conventional cultures typically takes 48–72 h. Identification of molecules measurable within a short time frame and closely associated with microbiologically confirmed VAP is therefore highly desirable. VAP is associated with significant influx of activated neutrophils into the alveolar space. We postulated that extracellular neutrophil proteases in bronchoalveolar lavage fluid (BALF) may reliably identify VAP in suspected cases.

**Methods** Fifty-four intubated and mechanically ventilated patients in the intensive care unit developed clinically suspected VAP and were recruited. Bronchoalveolar lavage (BAL) was performed using a standardised protocol. An aliquot of BALF was sent to the diagnostic microbiology laboratory for quantitative culture, with confirmation of VAP defined as growth of a pathogen(s) at  $>10^4$  colony forming units/ml. Remaining BALF was centrifuged. The following neutrophil-specific proteases were assayed in cell-free BALF supernatant—matrix metalloproteinase (MMP)-8 and MMP-9 by Luminex assay, and human neutrophil elastase (HNE) by enzyme-linked immunosorbent assay. Urea was simultaneously measured in serum and BALF, and used to correct for the dilution of epithelial lining induced by BAL. Receiver operating characteristic (ROC) curves were constructed and optimal specificity and sensitivity for each marker calculated.

**Results** Eleven patients (20%) had confirmed VAP. For HNE (cut off 670ng/ml) the ROC area under curve (AUC) was 0.87 ( $p<0.0001$ ), sensitivity 93%, specificity 79%. For MMP-8 (13 ng/ml), ROC AUC was 0.81 ( $p<0.005$ ), sensitivity 91%, specificity 63%. For MMP-9 (22 ng/ml), ROC AUC was 0.79 ( $p<0.005$ ), sensitivity 82%, specificity 63%.

**Conclusions** Neutrophil proteases are strongly associated with confirmed infection in cases of suspected VAP. The values for HNE, in particular, compare extremely favourably with any previously published equivalent values. These data suggest that neutrophil protease concentrations in BALF deserve further attention as potentially diagnostic markers for VAP. They further suggest that neutrophil proteases, inappropriately released into the extracellular space, may contribute to the pathophysiology of VAP.

## S80 THE ROLE OF MYD88 IN RHINOVIRUS 1B INFECTION

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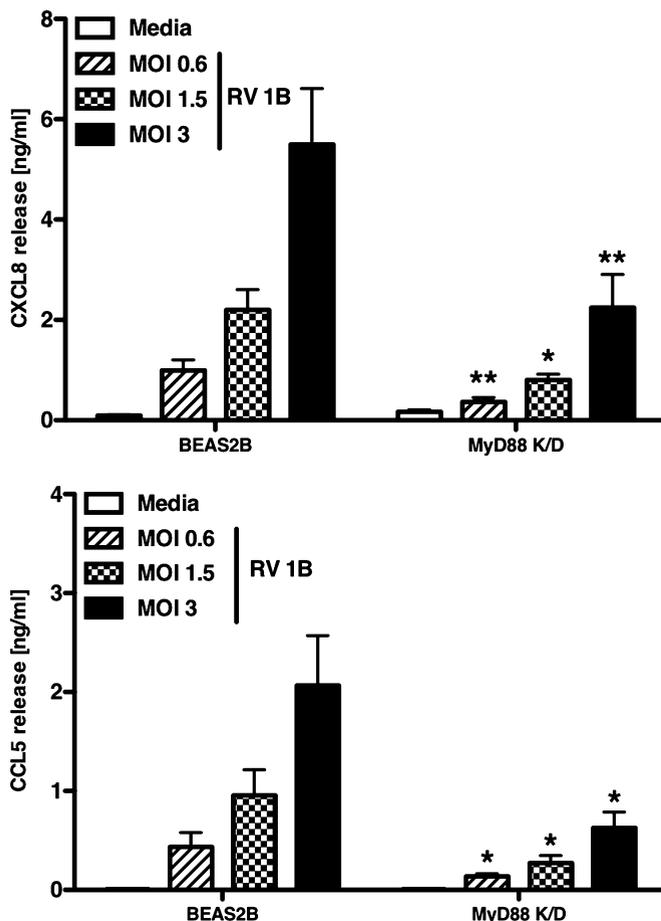
**Introduction** Rhinovirus (RV) are major triggers of acute asthma exacerbations and result in innate immune cell infiltration into the airways. Viral recognition by TLRs results in activation of pathways mediated by the adaptors MyD88 and TRIF, which predominantly control the production of proinflammatory cytokines and interferons respectively. We have previously shown that addition of the cytokine IL-1 (which also signals via MyD88) potentiates responses to the viral mimic Poly (I:C),<sup>1</sup> which acts in a MyD88-independent

manner. This demonstrates the potential for IL-1 signaling to impact on viral infection. In this study, we explored the ability of MyD88 to regulate responses to the natural viral pathogen, RV serotype 1B (RV-1B).

**Methods** MyD88 was stably knocked down in the immortalised bronchial epithelial cell line, BEAS-2B, using a lentiviral transduction system containing shRNA targeted to MyD88. Wildtype or MyD88<sup>KD</sup> cells were stimulated, in the presence or absence of human monocytes, with TNF $\alpha$  and IL-1 $\beta$ , poly(I:C), LPS and gardiquimod (TLR3, TLR4 and TLR7/8 agonists, respectively), or infected with RV-1B. Selected experiments were carried out in the presence of IL-1ra. Changes in cytokine release were measured by ELISA. Rates of viral replication were measured using quantitative PCR.

**Results** Costimulation of BEAS-2B cells with IL-1 and RV-1B caused a dramatic increase in proinflammatory (CXCL8), but not CCL5 production. MyD88<sup>KD</sup> cells with ~70% reduction in MyD88 mRNA levels showed no impairment to TNF $\alpha$  or poly(I:C) stimulation, but significantly reduced responses to IL-1 $\beta$ . MyD88<sup>KD</sup> cells also had significantly impaired responses to RV-1B as assessed by production of CXCL8 and CCL5. Inhibition of RV-induced CXCL8 production could also be achieved by pre-treatment with the IL-1 antagonist, IL-1ra. IL-1 $\beta$  was not produced from RV-infected cells, implicating other members of the IL-1 family in the response of epithelial cells to viral infection. Viral replication was more marked in MyD88<sup>KD</sup> cells.

**Conclusion** A reduction in MyD88 signalling modulates specific epithelial cell responses to rhinovirus, and thus may be an important target to control acute inflammation induced by human viral pathogens.



Abstract S80 Figure 1

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## REFERENCE

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## S81 PRIMARY TYPE II ALVEOLAR EPITHELIAL CELLS RESPOND DIFFERENTIALLY TO BACTERIAL VIRULENCE FACTORS

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**Introduction and objectives** The pathogens most commonly implicated in ventilator-associated pneumonia (VAP) are *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Although a florid inflammatory response characteristically occurs in the alveolar space in VAP, the underlying mechanisms remain unclear, partly owing to a lack of adequate models of alveolar injury. We therefore sought to characterise the response of primary human type II alveolar epithelial (ATII) cells to virulence factors from these pathogens.

**Methods** Primary ATII cells were derived from seven patients undergoing surgical resection for lung cancer. Lung tissue was refrigerated overnight; flushed with saline; incubated with trypsin; diced; incubated with DNase I; and strained/filtered. Macrophages and fibroblasts were removed by adherence. The resulting cell population was centrifuged, washed, resuspended and plated onto tissue culture plates pre-coated with type I bovine collagen at  $2 \times 10^6$  cells/ml. When cells achieved confluence medium was replenished and the following were added for 24 h: 100 ng/ml *P aeruginosa* lipopolysaccharide (LPS); 10 ug/ml *S aureus* lipoteichoic acid (LTA); 10 ug/ml *S aureus* peptidoglycan (PGN); 10 ng/ml human recombinant tumour necrosis factor alpha (TNF $\alpha$ ); or control medium. Supernatant was aspirated at 24 h and cytokines were measured by cytometric bead array.

**Results** Interleukin (IL)-1 $\beta$ , IL-6, IL-8 IL-10, IL-12p70 and TNF $\alpha$  were all detectable in control medium at 24 h. None of the measured cytokines were significantly altered by application of LPS or LTA. In contrast, PGN induced a significant rise in concentrations of IL-1 $\beta$ , IL-6, IL-8 IL-10 and TNF $\alpha$ . Addition of TNF $\alpha$  induced a significant increase in IL-6, IL-8 and IL-10. The only cytokine to be uniformly uninfluenced by stimulation was IL-12p70.

**Conclusions** In our hands primary ATII cells appeared to be unresponsive to *P aeruginosa* LPS or to *S aureus* LTA. By contrast, *S aureus* PGN provoked a brisk and significant inflammatory response simultaneously affecting a range of cytokines. These data suggest that ATII cells have strikingly different responses to individual bacterial virulence factors. They further suggest that PGN (but not LTA) contributes, at least in part, to the florid inflammatory response seen in Staphylococcal pneumonia.

## S82 SURVIVAL OF HIV-INFECTED PATIENTS ADMITTED TO THE INTENSIVE CARE UNIT

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**Background** Several studies from USA and Europe have suggested the outcome for HIV-infected patients admitted to the intensive care unit (ICU) has improved, concurrent with both the introduction of highly active antiretroviral therapy (HAART) and