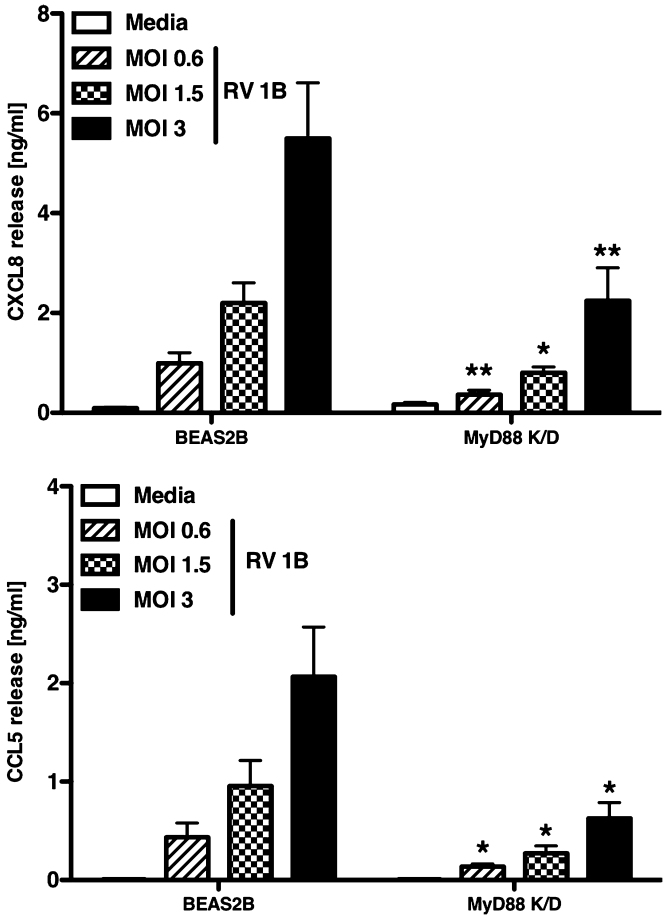
manner. This demonstrates the potential for IL-1signaling to impact on viral infection. In this study, we explored the ability of MyD88 to regulate responses to the natural viral pathogen, RV serotype 1B (RV-1B).

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 $MyD88^{KD}$ cells were stimulated, in the presence or absence of human monocytes, with TNFα and IL-1β, 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. $MyD88^{KD}$ cells with ~70% reduction in MyD88 mRNA levels showed no impairment to TNFα or poly(I:C) stimulation, but significantly reduced responses to IL-1β. $MyD88^{KD}$ 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-1β 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 $MyD88^{KD}$ 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

1. **Morris,** *et al***.** *FASEB* 2006; **20**:E1539–49.

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 2×10^6 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 (TNFα); or control medium. Supernatant was aspirated at 24 h and cytokines were measured by cytometric bead array.

Results Interleukin (IL)-1β, IL-6, IL-8 IL-10, IL-12p70 and TNFα 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-1β, IL-6, IL-8 IL-10 and TNFα. Addition of TNFa 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