co-cultured with ASM cells demonstrated CADM1 and CD117 co-localisation by confocal microscopy. CADM1 and CD117 expression on HLMC was closely co-localised at direct points of adhesion to ASM cells. In addition, immunoprecipitation of CADM1 resulted in the co-immunoprecipitation of CD117, also indicating the presence of a physical interaction.

In conclusion, our data support the view that adhesion of HLMC on ASM via CADM1 facilitates the close proximity of HLMC CD117 and ASM membrane-bound SCF and promotes mast cell ASM interactions.

### S114 MAST CELLS PROMOTE AIRWAY SMOOTH MUSCLE CELL DIFFERENTIATION VIA AUTOCRINE UPREGULATION OF TRANSFORMING GROWTH FACTOR BETA 1

L Woodman, S Siddiqui, G Cruse, A Sutcliffe, R Saunders, D Kaur, P Bradding, C Brightling. *University of Leicester, Leicester, UK*

Asthma is a major cause of morbidity and mortality worldwide. It is characterised by airway dysfunction and inflammation. A key determinant of the asthma phenotype is infiltration of airway smooth muscle bundles by activated mast cells. We hypothesised that interactions between these cells promotes airway smooth muscle differentiation into a more contractile phenotype.

In vitro co-culture of human airway smooth muscle cells with β-tryptase, or mast cells with or without IgE/anti-IgE activation, increased airway smooth muscle-derived transforming growth factor beta (TGF-β) 1 secretion, α-smooth muscle actin expression and agonist-provoked contraction. This promotion to a more contractile phenotype was inhibited by both the serine protease inhibitor leupeptin and TGF-β1 neutralisation, suggesting that the observed airway smooth muscle differentiation was driven by the autocrine release of TGF-β1 in response to activation by mast cell β-tryptase. Importantly, in vivo we found that in bronchial mucosal biopsies from asthmatic individuals the intensity of α-smooth muscle actin expression was strongly related to the number of mast cells within or adjacent to an airway smooth muscle bundle.

These findings suggest that mast cell localisation in the airway smooth muscle bundle promotes airway smooth muscle cell differentiation into a more contractile phenotype, thus contributing to the disordered airway physiology that characterises asthma.

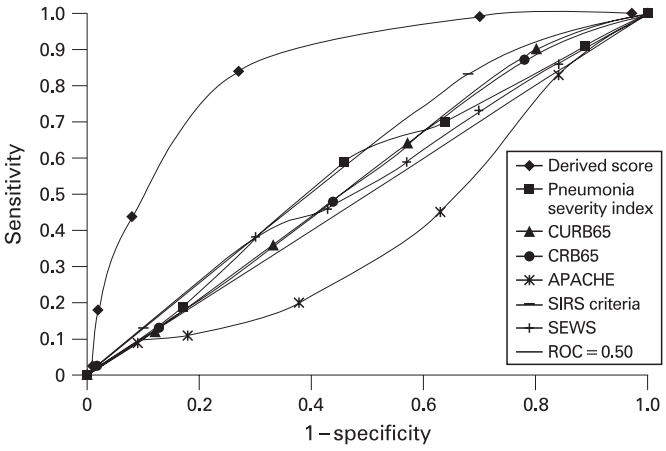
## Frontiers in lung infection

### S115 RISK FACTORS FOR THE DEVELOPMENT OF COMPLICATED PARAPNEUMONIC EFFUSIONS AND EMPYEMA IN COMMUNITY-ACQUIRED PNEUMONIA

JD Chalmers, A Singanayagam, C Scally, A Fawzi, MP Murray, AT Hill. *Royal Infirmary of Edinburgh, Edinburgh, UK*

**Background:** Complicated parapneumonic effusion and empyema (CPE/empyema) are serious complications of community-acquired pneumonia (CAP) associated with significant morbidity. The aim of this study was to identify key factors on admission predicting the development of CPE/empyema in patients admitted with CAP.

**Methods:** The authors conducted a prospective observational study of patients admitted with CAP. Multivariate logistic regression was used to evaluate admission factors that could predict the development of CPE/empyema including admission demographics, clinical features and laboratory tests. Pneumonia specific (PSI, CURB65 and CRB65) and generic sepsis scoring systems (APACHE II, SEWS, SIRS) were compared using the area under the receiver operator characteristic curve (AUC). Complicated parapneumonic effusion was defined according to the criteria described by Light and colleagues as pleural fluid pH <7.2, LDH >1000 iu/l, glucose <2.2 mmol/l. Empyema was defined as frank pus aspirated from the pleural space or positive Gram stain/culture for pathogenic organisms.




Abstract S115 Figure Receiver operator characteristic curves for prediction of complicated parapneumonic effusion or empyema.

**Results:** 1628 patients with respiratory infections were identified and ultimately 1269 patients with CAP were included in the study. 92 patients (7.2%) met the criteria for CPE/empyema. CURB65 (AUC 0.54) CRB65 (0.52), PSI (0.55), APACHE II (0.41), SIRS (0.57) and SEWS (0.53) had no value in CPE/empyema. Multivariate logistic regression identified albumin <30 g/l odds ratio (OR) 4.55 (2.45 to 8.45, p<0.001), sodium <130 mmol/l OR 2.70 (1.55 to 4.70, p = 0.005), platelet count >400 OR 4.09 (2.21 to 7.54, p<0.001), C-reactive protein >100 mg/l OR 15.7 (3.69 to 66.9, p<0.001) and a history of alcohol abuse OR 4.28 (1.87 to 9.82, p = 0.006) or intravenous drug use OR 2.82 (1.09 to 7.30, p = 0.03) as independently associated with the development of CPE/empyema. A history of chronic obstructive pulmonary disease was protective OR 0.18 (0.06 to 0.53, p = 0.002). A six-point scoring system using these combined variables was devised. In the presence of two or more of these factors the derived score had a sensitivity of 87%, specificity of 68.3%, positive predictive value of 17.7% and negative predictive value of 98.5%. The score had good discriminatory value AUC 0.84 (0.81 to 0.86, p<0.001). See fig.

**Conclusion:** This study has identified seven clinical factors predicting the development of CPE/empyema. Independent validation studies are needed.

### S116 EVIDENCE OF POLYMICROBIAL INFECTION IN NON-CYSTIC FIBROSIS BRONCHIECTASIS

JEM Stach, Z Zhao, CV Lanyon, J Perry, A De Soyza. *School of Biology, Newcastle University, Newcastle upon Tyne, UK*

**Background:** Data suggest that polymicrobial biofilms exist in the cystic fibrosis lung that may contribute to the exaggeration of illness or infection. It is unclear if polymicrobial biofilms are seen with other causes of bronchiectasis. Limitations in standard culture (overgrowth by other bacteria/resilience of microorganisms to standard culture techniques) mean standard culture may not detect the diversity of bacteria present in non-cystic fibrosis bronchiectasis. Current clinical culture techniques use an a priori decision on which organisms are likely to be present/important as causative infectious agents.

**Hypothesis:** Greater biodiversity will be found using non-culture-based techniques in bronchiectasis sputa compared with standard culture. 16S ribosomal RNA analysis will allow for the detection of bacteria that are resistant to culture.

**Methods:** Twenty-four sputa samples were collected from 21 adult bronchiectasis patients and analysed using standard culture. Total bacterial community from extracted nucleic acids was profiled using PCR density denaturing gel electrophoresis (DGGE) community profiling with band sequencing/in silico analysis. In vitro cultures.
**Results:** A range of pathogens was isolated from the sputa; in seven sputa no pathogens were isolated, *Pseudomonas aeruginosa* (PA 9), *Staphylococcus aureus* (SA 2), *Streptococcus pneumonia* (SP 2), *Haemophilus influenzae* (HI 2), *Moraxella* (Mx 2), *Serratia* 1 and *Proteus* (PR 1). In four sputa two pathogens were co-cultured; SP + PA, PR + coliform, HI + Mx and PA + HI. PCR–DGGE results: Preliminary analysis of 12 of the 24 sputa analysed indicate a complex bacterial community with 72 bacterial ribotypes identified allocated to 29 ribotype classes. Phylogenetic analysis reveals the presence of several pathogenic organisms not previously identified through culture based techniques and a far greater diversity of bacterial organisms than previously assumed.
**Conclusions:** The results to date indicate the possibility that non-cystic fibrosis bronchiectasis lungs are polymicrobial, with the detection of pathogens previously resistant to culture. These results are significant in the understanding and treatment of colonisation or subclinical infection in adult bronchiectasis patients. Better understanding of complex microbial communities may lead to atypical antibiotic combinations or novel approaches targeting biofilms.

## S117 INVASIVE CAPSULAR SEROTYPES OF *STREPTOCOCCUS PNEUMONIAE* ARE RELATIVELY RESISTANT TO COMPLEMENT COMPARED WITH NON-INVASIVE SEROTYPES

[1]CJ Hyams, [1]S Opel, [1]J Yuste, [2]WP Hanage, [3]B Henriques-Normark, [4]JN Wesier, [1]JS Brown, [2]BG Spratt. *[1]The Centre for Respiratory Research, UCL, London, UK; [2]Department of Infectious Disease Epidemiology, Imperial College, London, UK; [3]Smitt-Skydds Institute, Stockholm, Sweden; [4]Department of Pediatrics, University of Pennsylvania, Philadelphia, USA*

**Introduction:** The *Streptococcus pneumoniae* polysaccharide capsule is an essential virulence factor that varies in structure between capsular serotypes. Specific serotypes are known to be highly invasive, but it is currently unknown why these strains frequently cause infections whereas other serotypes are generally restricted to nasopharyngeal colonisation. As complement is a vital immune component to *S pneumoniae* and the capsule affects complement deposition, we hypothesised that capsular serotype-dependent differences in complement susceptibility may influence invasiveness. Therefore we have investigated: the relative importance of capsule and strain background for complement deposition on *S pneumoniae*; whether differences in complement sensitivity between serotypes correlate with invasiveness.
**Methods:** C3b/iC3b deposition on bacteria was measured in 25% human serum using flow cytometry for: isogenic *S pneumoniae* strains expressing capsular serotypes 4, 6A, 7F, 23F; four different clinical isolates with different genetic backgrounds for each of serotypes 4, 23F, 6A, and 14; further clinical isolates representing invasive and non-invasive serotypes.
**Results:** The relative fluorescence index (RFI) of C3b/iC3b deposition on isogenic strains varied with capsular serotype, with serotype 6A showing particularly high levels (848% of results for serotype 4). Results for sera deficient in specific complement components suggested these differences were mainly mediated by alternative pathway activity and independent of antibody levels. Some strains with different genetic backgrounds but the same capsular serotype also showed clear differences in C3b/iC3b deposition (eg, RFI for serotype 23F ST515 strain 8174 $\pm$ 490 vs 39321 $\pm$ 2293 for the ST277 strain). The median RFI C3b/iC3b deposition on 12 strains from invasive serotypes 1, 4 and 14 was 37890 (interquartile range (IQR) 16240–45409) compared with a median 65980 (IQR 37723–95143) for 18 strains of non-invasive capsular serotypes 9V, 6B, 23F and 19F ($p = 0.0118$).
**Conclusions:** C3b/iC3b deposition on *S pneumoniae* varies markedly between strains due to both capsular serotype but also due to other genetic differences. Invasive serotypes had lower levels of C3b/iC3b deposition than non-invasive strains and are therefore probably more resistant to complement-mediated immunity. To our knowledge these are the first data suggesting why some *S pneumoniae* strains are more likely to cause invasive disease than others.

## S118 ANTIMICROBIAL ACTIVITY OF HUMAN MAST CELLS TO PNEUMOCOCCI IS MEDIATED BY PNEUMOLYSIN

G Cruse, H Brewin, V Fernandes, J Salort, MS Marinas, PW Andrew, P Bradding, A Kadioglu. *University of Leicester, Leicester, UK*

Mast cells play a key role in allergic and inflammatory diseases. Whereas mast cell research is often centred on their deleterious pathological effects, they also play important roles in homeostatic physiology, with increasing evidence emerging for roles in both innate and adaptive immunity. Mouse mast cells have recently been shown to play a pivotal role in protection against bacterial infection, and cord blood-derived mast cells reduce bacterial viability in culture. We thus hypothesised that tissue-derived human lung mast cells (HLMC) would be protective against pneumococcal lung infection through direct antimicrobial activity.

HLMC markedly reduced the number of viable wildtype (WT) serotype-2 (D39) pneumococci in co-culture, but had no effect on the viability of an isogenic pneumolysin-deficient (PLN-A) pneumococcus. In addition, despite evidence of phagocytosis of both WT and PLN-A pneumococci by mast cells, the majority of this antimicrobial effect did not require cell–cell contact since separation by a membrane did not eliminate WT pneumococcal lysis, suggesting that mast cells release antimicrobial agents upon activation with pneumolysin. We therefore studied the effects of WT and PLN-A pneumococci on mast cell mediator release and found that WT, but not PLN-A, pneumococci induced the release of $LTC_4$ from mast cells in a dose-dependent manner, which was not accompanied by histamine release. In addition, stimulation of mast cells with sublytic concentrations of purified pneumolysin replicated this effect. Interestingly, WT pneumococci reduced HLMC viability in a dose-dependent manner after 1 h of incubation, which was more evident after 6 h. As pneumolysin is a key virulence factor of pneumococci, we next tested the effect of the pneumolysin deficient (PLN-A) isogenic mutant. PLN-A also reduced HLMC viability although to a lesser degree, suggesting that pneumolysin is only partly responsible for HLMC cytotoxicity. Indeed, a cytotoxicity assay with pneumolysin revealed that HLMC were resistant to lysis by pneumolysin.

In summary, HLMC resident in the airways and lung parenchyma are endowed with direct antimicrobial capability and release pro-inflammatory mediators, which have the ability to recruit professional phagocytes. This unusual phenomenon of specific generation of newly formed mediators without the release of preformed granule-stored mediators is consistent with the activation of TLR-4 by pneumolysin.

## S119 GALECTIN-3 REDUCES THE SEVERITY OF PNEUMOCOCCAL PNEUMONIA BY AUGMENTING NEUTROPHIL FUNCTION

[1]SL Farnworth, [1]NC Henderson, [1]AC Mackinnon, [1]KM Atkinson, [1]T Wilkinson, [1]K Dhaliwal, [2]K Hayashi, [1]AJ Simpson, [1]AG Rossi, [1]C Haslett, [1]T Sethi. *[1]University of Edinburgh, Edinburgh, UK; [2]Sendai City Medical Centre, Sendai City, Japan*

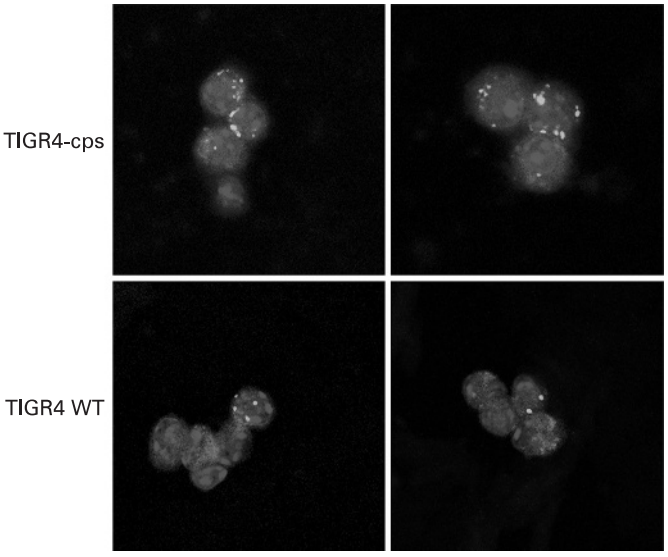
*Streptococcus pneumoniae* is the leading cause of community-acquired pneumonia worldwide, resulting in high mortality. Galectin-3 (gal-3), a beta-galactoside binding lectin implicated in many facets of the

inflammatory response, accumulates in the lungs of mice infected with *S pneumoniae* correlating with the onset of neutrophil extravasation. We tested the hypothesis that gal-3 reduces the severity of pneumococcal pneumonia by augmenting neutrophil function using both in vivo and in vitro techniques. In vivo, gal-3 deficient (gal-3-/-) mice develop more severe pneumonia following *S pneumoniae* infection, as demonstrated by increased bacteraemia and lung damage compared with wild-type mice. In vitro we show that gal-3 directly acts as a neutrophil-activating agent and potentiates the effect of fMLP, exogenous gal-3 augments neutrophil phagocytosis of bacteria and delays neutrophil apoptosis, phagocytosis of apoptotic neutrophils by gal-3-/- macrophages is less efficient compared with wild type, gal-3 demonstrates bacteriostatic properties against *S pneumoniae*. Furthermore, add-back of recombinant gal-3 in vivo protects gal-3-/- mice from developing severe pneumonia. Together, these results demonstrate that gal-3 is a key molecule in the host defence against pneumococcal infection. Therapeutic strategies designed to augment gal-3 activity may both enhance inflammatory cell function (by directly affecting neutrophil responsiveness and prolonging neutrophil longevity) and have direct bacteriostatic activity, improving clinical outcomes after severe pneumococcal infection.

### S120 THE *STREPTOCOCCUS PNEUMONIAE* CAPSULE IS ESSENTIAL FOR EVASION OF ALVEOLAR MACROPHAGE-MEDIATED PULMONARY IMMUNITY

[1]CJ Hyams, [1]EM Camberlein, [1]J Yuste, [2]M Noursadeghi, [1]C Michael, [3]JN Weiser, [1]JS Brown. *[1]The Centre for Respiratory Research, UCL, London, UK; [2]Department of Immunology and Molecular Pathology, UCL, London, UK; [3]Departments of Microbiology and Pediatrics, University of Pennsylvania, Philadelphia, USA*

**Introduction:** Although the *Streptococcus pneumoniae* capsule is known to be an essential virulence factor, there are surprisingly few data on how the capsule prevents pneumonia. We have therefore investigated the effects of the *S pneumoniae* capsule on complement-dependent phagocytosis and on interactions with alveolar macrophages (AM) in a mouse model of pneumonia.
**Methods:** Using encapsulated and genetically engineered unencapsulated *S pneumoniae* strains from two serotypes (2 and 4) the following experiments were performed using flow cytometry and confocal microscopy: C3b/iC3b deposition on bacteria in human serum; phagocytosis by a macrophage cell line and neutrophils extracted from human volunteers (using cytochalasin D and trypan blue to differentiate between phagocytosis and adherence to cell surfaces); phagocytosis by mouse AM 6 h post-intranasal inoculation of bacteria into wild-type and complement-deficient mice. In addition the rate of clearance of bacteria from the lungs was assessed.
**Results:** C3b deposition was markedly increased on unencapsulated compared with encapsulated strains (eg, relative fluorescence index (RFI) of C3b deposition of 211 130 ± 19 227 vs 11 378 ± 1814, respectively, for the serotype 4 strain). Unencapsulated bacteria were more readily phagocytosed by neutrophils than encapsulated bacteria (proportion of neutrophils associated with bacteria 49 ± 1.56 vs 17 ± 0.6, respectively, for the ST4 strain) and these differences were mainly complement dependent. Furthermore, unencapsulated bacteria stimulated greater nuclear transfer of nuclear factor kappa B (NFκB) in macrophages than encapsulated bacteria (median of nuclear : cytoplasmic ratio for unencapsulated of 4.096 (interquartile range (IQR) 3.32–4.664) vs 2.943 (IQR 2.719–3.122) for encapsulated strain). Within 6 h of intranasal inoculation there was a marked increase in phagocytosis by AM of the unencapsulated (RFI of 2498 ± 615) compared with encapsulated (RFI 9349 ± 3404) (fig), and this was associated with rapid clearance of unencapsulated bacteria (>3 $\log_{10}$ fewer unencapsulated bacteria for the serotype 4 strains) from lavage fluid. Interestingly, repeated experiments with complement-deficient mice demonstrated that the effect of the capsule on interactions with AM was only partly complement dependent.
**Conclusions:** These data demonstrate that capsule is vital for *S pneumoniae* evasion of early pulmonary immune responses by inhibiting complement-dependent and independent interactions with AM.




Ex vivo alveolar macrophages from CD1 mice inoculated intra-nasally with FAM-SE labelled bacteria and harvested after 4 h (red = F4/80, blue = Dapi, green = FAM-SE)

Abstract S120 Figure

## Clinical investigation of pulmonary vascular diseases

### S121 COMPUTERISED TOMOGRAPHY PULMONARY ANGIOGRAPHY: SIGNIFICANT SECONDARY FINDINGS AND QUANTIFYING THE ADDED BURDEN TO RESOURCES

[1]B Barker, [1]V Rao, [2]R Rao, [1]R Manns, [1]K Srinivasan, [1]H Moudgil. *[1]Shrewsbury and Telford Hospitals NHS Trust, Telford, UK; [2]University of Birmingham, Birmingham, UK*

**Introduction and Objectives:** Paralleling the increased use of computerised tomography pulmonary angiography (CTPA) in the investigation of pulmonary thromboembolsm (PTE) is anecdotal evidence that there has been an increased subsequent demand particularly on radiology resources as a result of incidental or co-lateral findings. Presently we set out to quantify and investigate the nature of these investigations with an evaluation of return on investment.
**Methods:** All 252 CTPA undertaken over a 12-month period to 31 December 2007 for primary exclusion of PTE were identified. The median age of patients was 69 years (range 18–98) with 148 (59%) women. Supporting data were gathered retrospectively from the hospital PACS radiology reporting system, from e-script electronic result reports and discharge summaries.
**Results:** Although 83/252 (33%) had confirmed PTE on CTPA, from the group as a whole (n = 252), additional diagnoses other than PTE were also reported in 136 (54%) patients and in whom for 91/252 (36%) this was a new and significant finding. Comparatively, more of these additional outcomes were reported in the group with no PTE (103/169 (61%) vs 33/83 (40%), p = 0.002). Analysed together, these additional findings included consolidation (10%), emphysema (9%), primary lung carcinoma