with FK506 inhibits maturation in this context. This suggests an inhibitory effect of FK506 on innate antigen presentation to T-cells and may impair the adaptive immune response to invasive aspergillosis in lung transplants recipients.

REFERENCE

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S84 SPUTUM NEUTROPHILS BUT NOT INTERLEUKIN-8 (IL-8) OR INTERLEUKIN 17 (IL-17) CORRELATE WITH THE BRONCHIECTASIS SEVERITY INDEX (BSI)

[1]S Koustas, [1]A Peel, [1]J Scott, [2]J Davison, [3]K Jiwa, [1]S Carnell, [1]AJ Simpson, [1]A De Soyza. *[1]Newcastle University, Newcastle Upon Tyne, UK; [2]Adult Bronchiectasis Service, Freeman Hospital, Newcastle Upon Tyne, UK; [3]Sir William Leech Centre for Lung Research, Newcastle Upon Tyne, UK*

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Background Bronchiectasis is a progressive neutrophilic inflammatory lung disease associated with abnormal local cytokine release with possible systemic overspill. Early data suggests that interleukin-17 (IL-17) could be involved in the enhanced infiltration of neutrophils in the lungs, via the induction of IL-8 release, and has emerged as a possible biomarker for other chronic neutrophilic lung diseases.

Aims

1. to investigate the potential use of IL-17 and IL-8 as biomarkers of disease severity in bronchiectasis by utilising a multidimensional clinical severity scoring system, the Bronchiectasis Severity Index (BSI).
2. correlate sputum neutrophils and pathogen status with serum or sputum IL-17 and IL-8 levels.

Methods Spontaneous sputa and sera were collected from stable adult bronchiectasis patients attending a specialist clinic. We quantified both IL-17 and IL -8 concentrations in the pulmonary compartment (sputum) and the systemic compartment (serum) of 119 stable bronchiectasis patients and 26 healthy volunteers. Sputum neutrophils were conducted using standard methods.

Results The mean patient age was 65 years, with 24% in mild BSI, 39% moderate BSI and 46% (43% idiopathic, 24% post infectious). IL-17 in the sputum of bronchiectasis patients was found to be two-fold greater than in serum suggesting "local" release (10 pg/ml vs 5 pg/ml). Statistical analysis revealed a significant correlation between these two variables, suggesting a "spillover" of cytokines from the lungs ($p < 0.001$).

However, there was no significant difference in serum IL-17 levels between bronchiectasis and healthy subjects (0 ± 2 pg/ml). In addition, no significant correlation was found between IL-8 and IL-17 levels in the sputum of patients. Sputum IL-17 levels were found to have a significant negative correlation with BSI severity scoring, but this was not reproduced when individual BSI parameters were analysed. IL-8 similarly performed poorly in correlating with BSI. In contrast more severe BSI scores were significantly associated with higher% neutrophils in sputa ($p = 0.045$).

Conclusions The clinical utility of IL-17 and IL-8 as biomarkers for the prediction of disease severity in bronchiectasis patients appears poor. These data may also suggest targeting these chemokines are of limited value. Focus in bronchiectasis may need shifted from neutrophil chemokines to factors that inhibit apoptosis and/or promote neutrophil persistence in the airway.

S85 PNEUMOLYSIN TRIGGERS THE PRODUCTION OF PLATELET-ACTIVATING FACTOR BY HUMAN NEUTROPHILS IN VITRO

[1]R Anderson, [1]JG Nel, [1]AJ Theron, [2]TJ Mitchell, [3]C Feldman. *[1]Departments of Immunology and Haematology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa; [2]School of Immunity and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK; [3]Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*

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Introduction and objectives Pneumolysin (Ply), the major protein virulence factor of the pneumococcus, has been implicated in the pathogenesis of acute lung injury and acute coronary events, both of which are significant causes of mortality, in severe pneumococcal disease. However, the role of Ply in promoting neutrophil/platelet cross-talk, increasingly recognised as a key event in the immunopathogenesis of inflammation-mediated pulmonary and cardiovascular damage is unknown. This issue has been addressed in the current study, which is focused on the effects of exposure of isolated human blood neutrophils to Ply on the production of the platelet-targeted, pro-inflammatory lipids, platelet-activating factor (PAF) and thromboxane A_2 (TXA_2).

Methods Neutrophils, isolated from the blood of healthy, adult humans, were suspended at a concentration of 2×10^6/ml in Hanks balanced salt solution and preincubated for 10 min at 37°C followed by addition of recombinant Ply (5–80 ng/ml), or the pneumolysoid, delta6Ply (80 ng/ml, negative control), or the calcium ionophore, A23187, (2 μM, positive control). After 5 min of incubation, the reactions were terminated and PAF and TXA_2 assayed in the cell-free supernatants using sandwich ELISA procedures.

Results These are shown in the accompanying Table 1. Exposure of neutrophils to Ply resulted in dose-related enhancement of production of PAF, which achieved statistical significance at concentrations ≥20 ng/ml of the toxin, while delta6Ply was ineffective, and A23187 extremely potent. Similar, but less impressive effects were noted in the case of TXA_2.

Abstract S85 Table 1

Agent	PAF (pg/ml)	TXA_2 (pg/ml)
Control	4.5 ± 1.2	13.5 ± 1.4
Ply, 20 ng/ml	9.6 ± 2.4*	16.1 ± 2.0*
Ply, 40 ng/ml	12.1 ± 3.5*	17.7 ± 2.1*
Ply, 80 ng/ml	13.1 ± 4.0*	17.7 ± 2.3*
delta6Ply, 80 ng/ml	5.4 ± 1.0	12.3 ± 1.5
A23187, 2 μM	37.0 ± 5.3*	28.0 ± 1.4*

*$p < 0.05$-$p < 0.002$.

Conclusion Ply, via its pore-forming activity, activates the production of PAF and, to a lesser extent, TXA_2, by neutrophils, potentially augmenting pro-inflammatory cross-talk between these cells and platelets, an activity of the toxin which may contribute to the immunopathogenesis of lung and cardiac injury in severe pneumococcal disease.

S86 THE ANTI-INFLAMMATORY EFFECTS OF PNEUMOLYSIN

JN Periselneris, T James, M Noursadeghi, JS Brown. *University College London, London, UK*

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The inflammatory response to bacteria requires the interaction of pattern recognition receptors with bacterial surface constituents, and humans deficient in components of inflammatory signalling pathways such as IRAK4 are prone to invasive pneumococcal disease. Pneumolysin is a well-recognised virulence factor for Streptococcus pneumoniae that has multiple effects on the host immune response that are primarily thought to be pro-inflammatory; including causing IL1β release due to pore formation, and epithelial cell layer breakdown. We hypothesised that pneumolysin deficient TIGR4 (a serotype 4 strain) would induce less inflammatory cytokines than wildtype from human monocyte derived macrophages. While both pore forming and non-cytolytic purified pneumolysin induced dose dependent inflammatory cytokine release, the pneumolysin deficient bacteria induced greater TNF and IL6 than wildtype, by qPCR and ELISA measurement of protein. This was reduced by inhibition of phagocytosis with cytochalasin D. Given the pore forming effects of pneumolysin we assessed whether differential cell death contributed to the differences in inflammatory response. While wildtype bacteria caused more cell death at 24 h, inhibition of caspases had no effect on the cytokine response suggesting that apoptosis pathways don't directly influence the early inflammatory response. Transcriptome analysis confirmed increased pro-inflammatory and interferon gene signalling with the mutant strain, with reduction of the inflammatory and interferon signature with inhibition of phagocytosis. Wildtype bacteria induced less NFκB translocation, but more IRF3 translocation than Δ*ply*. An *in vivo* intranasal mouse infection showed wildtype was more virulent, with more bacteria recovered from bronchoalveolar lavage fluid at 4 h. However, this was associated with reduced TNF compared to Δ*ply*. Neutralising TNF intranasally abrogated the difference in bacteria recovered between wildtype and Δ*ply*. Thus, the early inflammation dampening effects of pneumolysin released within the phagolysosome may be an important contribution to its virulence by allowing bacterial replication at mucosal surfaces. This may be due to IRF3 mediated inhibition of inflammatory cytokine transcription. Better understanding of the biology of pneumolysin may aid in adjuvant treatment of *S. pneumoniae*.

New developments in cough

S87 ARE ORAL STEROIDS EFFECTIVE IN TREATING THE SYMPTOMS OF ACUTE LOWER RESPIRATORY TRACT INFECTION IN NON-ASTHMATIC ADULTS? THE ORAL STEROIDS FOR ACUTE COUGH (OSAC) PLACEBO-CONTROLLED RANDOMISED TRIAL

[1]AD Hay, [1]HE Downing, [1]ST Brookes, [1]G Young, [2]A Harnden, [1]SP Hollinghurst, [3]D Kendrick, [4]P Little, [1]MT May, [4]MV Moore, [3]E Orton, [2]K Wang, [5]MJ Thompson. *[1]University of Bristol, Bristol, UK; [2]University of Oxford, Oxford, UK; [3]University of Nottingham, Nottingham, UK; [4]University of Southampton, Southampton, UK; [5]University of Washington, Seattle, USA*

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Background The majority of UK adults experience at least one lower respiratory tract infection (LRTI, or acute bronchitis) a year. Despite an absence of evidence in this patient group, some GPs prescribe inhaled or oral corticosteroids. OSAC sought to demonstrate 'proof of concept' symptomatic effectiveness of a moderate dose of oral corticosteroid for adults without asthma or COPD with acute LRTI.

Methods OSAC was a double blind, placebo controlled RCT set in GP practices in England, powered to investigate if oral prednisolone reduces the duration of moderately bad or worse cough and/or the severity of its associated symptoms, when compared to placebo, by at least 20%. Adults (≥18 years) with acute (≤28 days) cough, for whom same-day antibiotics were not clinically indicated, and without asthma or COPD, received 40 mg oral prednisolone or matched placebo for 5 days. Symptom diaries, completed for up to 28 days, measured two primary outcomes: the duration of moderately bad or worse cough; and the average severity of all symptoms on days 2 to 4 on a scale of 0–6. We sought to demonstrate a minimum clinically important reduction of 20% in each outcome.

Results 398 participants were randomised to either prednisolone or placebo tablets (198 and 200 respectively) from 54 UK primary care sites. Attrition was lower than expected, giving over 85% power for the two primary outcomes. Data were analysed on an intention-to-treat basis. The median duration of moderately bad or worse cough was 5 days in both groups (IQRs 2–8 and 3–8 for prednisolone and placebo respectively). Adjusting for trial centre and baseline characteristics, this gave a hazard ratio of 1.11 (95% CI 0.89 to 1.39, p = 0.35). Symptom severity was lower in the prednisolone group (mean 1.99 vs 2.16), adjusted difference -0.090 (-0.212 to 0.003, p = 0.152).

Conclusions We found no evidence that a moderately high dose of oral corticosteroid reduced either duration of moderately bad (or worse) cough, or symptom severity at days 2 to 4 in adults without asthma or COPD with LRTI not requiring immediate antibiotic treatment. Lower dose oral or high dose inhaled corticosteroids are also unlikely to be beneficial.

S88 THE VIRAL MIMIC POLYINOSINIC: POLYCYTIDYLIC ACID (POLY I:C) INDUCES TRPA1 CHANNEL HYPER-RESPONSIVENESS IN AN ADULT HUMAN STEM CELL-DERIVED SENSORY NEURONAL MODEL

[1]R Clarke, [1]K Monaghan, [2]I About, [1]I El Karim, [1]JG McGeown, [1]SL Cosby, [1]TM Curtis, [1]FT Lundy, [1]L McGarvey. *[1]School of Medicine, Dentistry and Biomedical Sciences, Queen's University, Belfast, Northern Ireland, Belfast, N Ireland; [2]Aix Marseille Université, Marseille, France*

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Background Changes in airway neuronal activity are likely to underpin the heightened irritant responses such as excessive cough and wheeze which accompany respiratory virus induced exacerbations of airways disease. The mechanisms responsible are unknown but we hypothesised that neurons express pathogen recognition receptors such as toll-like receptors (TLR) through which viruses may alter neural function. Investigating this is hampered by the lack of suitable human tissues with both nerve endings and cell bodies present. We have refined an adult human neural crest stem cell-derived sensory neuronal model to overcome this obstacle.

Methods Human dental pulp stem cells (hDPSCs) were differentiated towards a neuronal phenotype, termed peripheral neuronal equivalents (PNEs). Using molecular and immunofluorescent techniques, together with whole cell patch clamp electrophysiology, we investigated the expression and function of TLRs and the transient receptor potential (TRP) channels TRPV1 and TRPA1 on PNEs. We then assessed the effects of exposure to a viral mimic, the synthetic TLR3 agonist (polyI:C), on cytokine