

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

1 Herbst S, Shah A, Mazon Moya M, et al. *EMBO Mol Med*. 2015;7(3):240–58

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

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

10.1136/thoraxjnl-2015-207770.90

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

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

10.1136/thoraxjnl-2015-207770.91

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 A₂ (TXA₂).

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 TXA₂ 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 TXA₂.

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, TXA₂, 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

10.1136/thoraxjnl-2015-207770.92