WS19.1 Inflammation and oxidation biomarkers in patients with cystic fibrosis (CF): azithromycin influence
C. Olivera1, M.R. Jimeno Galván2, A. Dorado Galindo3, F. Esplidora Hernández3, E. Rubio Martín3, E. Doña Diaz4, V. Contraser Bolivar3, G. Olivera3, 1Málaga University. Regional University Málaga Hospital, CMU Endocrinology and Nutrition and Respiratory Diseases, Málaga, Spain; 2Punta Europa Hospital, Medicine Services Pneumology Unit, Algeciras, Spain; 3Regional University Málaga Hospital, CMU Endocrinology and Nutrition and Respiratory Diseases, Málaga, Spain

Objectives: Macrolides appear to modulate the inflammatory response in Cystic fibrosis (CF) patients and could influence oxidative stress. The objective was to assess levels of inflammation and oxidation biomarkers and evaluate whether there is an association with the intake of macrolides.

Methods: A cross-sectional descriptive study. Clinical and radiological severity parameters were collected (exacerbations, Bailla scoring system, amount of sputum, spirometry) and inflammatory (interleukin-6, TNF-α and CRP) and oxidative stress (total antioxidant capacity [TAC], catalase [CAT] activity, superoxide dismutase [SOD] activity, thioglutathione peroxidase [GPx] activity, thiobarbituric acid reactive substances [TBARs, lipid peroxidation] and Isoprostanates) activity markers were measured.

Results: 36 clinically stable patients (mean age 27.8 ± 9.4) were enrolled. Baseline data were compared according to TAC levels (TAC< 10 vs. TAC>10). CF subjects had significantly higher levels of IL-6, TNF-α and CRP TBARs, Isoprostanates and lower SOD activity than the controls. Patients who were treated with azithromycin had a clinically more severe disease (greater number of exacerbations, worse Bailla score, 5%FEV1, higher percentage of Homozygous F508del, higher percentage of chronic colonization), despite which they presented significantly lower levels of TNF-α (3.1 ±0.2 vs. 4.4±2.2, P<0.05). There were no differences in the other parameters analysed.

Conclusion: Use of azithromycin appears to modulate inflammatory response in CF patients and is associated with a decrease in TNF-alpha levels at systemic level without changes in oxidation parameters.

WS19.2 Novel immunological tests for detection of Mycobacterium abscessus infection in patients with cystic fibrosis
D. Schramm1, V. Nkwoano1, M. Steindor1, B. Uebenberg3, M. Jacobson1, 1University Children’s Hospital Duesseldorf, Paediatric Pulmonology, Duesseldorf, Germany

Objectives: Infections with non-tuberculous mycobacteria, especially Mycobacterium abscessus (MABSC) are frequently observed in patients with cystic fibrosis (CF). MABSC can cause chronic infection with severe clinical manifestations. So far no immunological or serological tests for the detection MABSC infections are available.

Methods: The aim of this study is to establish a specific immunological test for the detection of MABSC infections in children with CF. Therefore we performed whole blood in vitro stimulation with purified protein derivates (PPD) of MABSC (Abscessin) to determine T-cell immunity against previous and current mycobacterial infections in CF patients and age-matched controls. Due to cross-reactivity of anti-mycobacterial immunity and to distinguish immunity against other possible mycobacterial infections, PPDs of M. avium (Sensitin) and M. tuberculosis (tuberculin) have been used concomitantly. Cytokines as well as the phenotype of MABSC specific T cells were determined by flow cytometry. All CF patients underwent extensive microbiological tests for NTM according to the ATS criteria.

Results: Initial results of CF patients (n=28) revealed four confirmed NTM-positive cases who had positive immune responses against MABSC. Of 24 CF patients with so far negative NTM cultures, ten showed immunity against NTM. In these cases previously eradicated NTM infections may have occurred. Ongoing studies aim at establishing NTM-specific PCR analysis from sputum to exclude false negative NTM cultures as another possible explanation for divergent result. In addition phenotypic T-cell characterization will reveal whether acute and previous NTM infection can be distinguished.

WS19.3 Virulence of serial Pseudomonas aeruginosa isolates grown under aerobic and anaerobic conditions using the Galleria mellonella infection model
E. Vallesayas1, L. Sherrard1, D. McLean2, D.G. Downey1, M. Tunney1, J.S. Elborn3, 1Queen’s University Belfast, CF & Airways Microbiology Research Group, Belfast, United Kingdom; 2Belfast Health and Social Care Trust, Adult CF Centre, Belfast, United Kingdom

Objectives: Increased P aeruginosa (PA) virulence may contribute to pulmonary exacerbations in CF. The aims of this ongoing study are to (1) investigate the impact of culture conditions (aerobic vs. anaerobic) on in vivo PA virulence and (2) determine if there is a difference in the virulence of PA cultured from CF patients during periods of clinical stability and infective exacerbation.

Methods: PA (n=8) recovered at 2 different time points from 1 CF patient, when clinically stable, and a control strain (PA206), were cultured for 48h from each time point. Each isolate was grown under aerobic and anaerobic conditions. Batches of Galleria mellonella larvae (n=10) were infected with each isolate ( inoculum of 10^5 CFU/ml). Virulence was determined by calculating percentage larvae survival and haemocyte (homologue of neutrophils) density at 24 hours.

Results: Survival (X±S.D.): Aerobic Anaerobic Isolate 1 30 10 Isolate 2 30 10 Isolate 3 80 60 Isolate 4 20 0 Isolate 5 30 10 Isolate 6 90 80 Isolate 7 0 0 Isolate 8 0 0 Control (PA206) 0 0

Conclusion: These results suggest that PA is more virulent to larvae when grown anaerobically. Lower haemocyte density is hypothesised to be the result of premature cell lysis due to overwhelming infection within larvae. Ongoing work includes investigating if PA virulence changes during exacerbations and molecular typing to characterise the strains and their virulence genes.

Work supported by AMMI Canada/Pfizer Post-Residency Fellowship and CF Canada.

WS19.4 Pro-inflammatory response of THP-1 monocytic cells to lipopolysaccharide from Pseudomonas aeruginosa isolated from CF patients
T. Matteri1,2, A. Glasgow1, A. Hanuszkiwiecz1, M. Valvano1, A. Stilgoe1, C. Taggart1, S. Weldon1, J.S. Elborn1, M. Tunney1, 1Queen’s University Belfast, School of Pharmacy, Belfast, United Kingdom; 2CF & Airways Microbiology Research Group, Queen’s University Belfast, Belfast, United Kingdom; 4Department of Chemical Sciences, University of Napoli Federico II, Napoli, Italy

Objectives: Bacterial LPS can elicit a potent response from the eukaryotic innate immune system and is recognised by host receptors such as TLR-4. The aim of this study was to determine the effects of Pseudomonas aeruginosa LPS on pro-inflammatory cytokine production by THP-1 monocytic cells.

Methods: LPS from a P. intermedia type strain and a CF clinical P. denticola isolate were used. LPS was included as a control. THP-1 cells were stimulated with purified P. intermedia LPS (10μg/ml), unpurified P. denticola LPS (2μg/ml), and E. coli LPS (100ng/ml) for 3, 6, 12, and 24 hours. Pro-inflammatory cytokines in cell free supernatants were assessed and cell viability determined at each time point.

Results: Peak THP-1 cell pro-inflammatory response to both P. intermedia and E. coli LPS was of a similar order of magnitude with regard to 4 pro-inflammatory cytokines.

Table: THP-1 cell peak response post stimulation with LPS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytokine levels (pg/ml)</th>
<th>Median (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli LPS</td>
<td>IL-8 8397 (105.3)</td>
<td>162 (4.6)</td>
</tr>
<tr>
<td></td>
<td>IL-10 755.9</td>
<td>14 (3.1)</td>
</tr>
<tr>
<td>P. intermedia LPS</td>
<td>9006 (775.9)</td>
<td>87 (2.7)</td>
</tr>
<tr>
<td></td>
<td>IL-10 24 (0.7)</td>
<td>14 (1.3)</td>
</tr>
<tr>
<td>P. denticola LPS</td>
<td>1662 (162.8)</td>
<td>34 (0.7)</td>
</tr>
<tr>
<td></td>
<td>IL-10 6 (0.3)</td>
<td>10 (0.9)</td>
</tr>
</tbody>
</table>

Conclusion: The results suggest that P. intermedia LPS is more potent at stimulating pro-inflammatory cytokines compared to E. coli LPS. Further work is needed to determine the mechanisms by which these differences occur.

Work supported by the NIHR-Belfast Bio-Immunology Research Centre (BRIC), the N€˜orwegian Research Council (Project 231748) and the University of Glasgow.