WS19.1 Inflammation and oxidation biomarkers in patients with cystic fibrosis (CF): azithromycin influence

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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, Bahlla 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, glutathione peroxidase [GPx] activity, thiobarbituric acid reactive substances [TBARs, lipid peroxidation] and Isoprostanes) activity markers were measured.
Results: 36 clinically stable patients (mean age 27.8) and 41 controls of similar age, sex and BMI were recruited. 23 (63.8%) patients were treated with azithromycin. CF subjects had significantly higher levels of IL-6, TNF-α and CRP, TBAs, isoprostanes and lower SOD activity than the controls. Patients who were treated with azithromycin had a clinically more severe disease (greater number of exacerbations, worst Bahlla score, %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

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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* restimulation with purified protein derivate (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 routine 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

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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 one CF patient, when clinically stable, and a control strain (Pa01) have been studied. Each isolate was grown under aerobic and anaerobic conditions. Batches of *Galleria mellonella* larva (n = 10) were infected with each isolate (inoculum of 10^7 CFU/ml). Virulence was determined by calculating percentage larvae survival and haemocyte (homologue of neutrophils) density at 24 hours.
Results: Survival ($X^2 = 17.827$, $P < 0.001$, McNemar's test, Table) and haemocyte density ($P = 0.031$, Wilcoxon signed rank test) were lower when larvae were infected with PA grown anaerobically.

Table: Larvae survival at 24 hours following infection

	Survival (%)	
	Aerobic	Anaerobic
Isolate 1	0	10
Isolate 2	50	10
Isolate 3	30	40
Isolate 4	80	60
Isolate 5	20	0
Isolate 6	30	10
Isolate 7	90	80
Isolate 8	0	0
Control (Pa01)	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 characterize the strains and their virulence genes.
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WS19.4 Pro-inflammatory response of THP-1 monocytic cells to lipopolysaccharide from *Prevotella* sp. isolated from CF patients

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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 *Prevotella* spp. 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. TLR grade *E. coli* LPS was included as a control. THP-1 cells were stimulated with purified *P. intermedia* LPS (10 ng/ml), unpurified *P. denticola* LPS (2 μg/ml), and *E. coli* LPS (100 ng/ml) for 3, 6, 12, and 24 hours. Pro-inflammatory cytokines in cell free supernatants were assayed and cell viability determined at each time point.
Results: Peak THP-1 cell pro-inflammatory response to both *Prevotella* spp. 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

	Cytokine levels (pg/ml), mean (SD)			
Treatment	IL-8	IL-1β	IL-6	TNF-α
P. intermedia	8397 (303.5)	162 (4.6)	14 (1.3)	3863 (276.9)
E. coli	9006 (775.9)	71 (0.1)	42 (0.7)	1199 (1.3)
P. denticola	1662 (162.8)	34 (0.7)	10 (0.9)	851 (11.3)
Unstimulated	10 (0.3)	Not detected	0.5 (0.2)	2.9 (0.2)

Peak values for IL-8, IL-1β, and IL-6 occurred at 6–12 hours in response to *Prevotella* spp. LPS, and at 24 hours in response to *E. coli* LPS.
Conclusions: The presence of *Prevotella* spp. within the CF lung may, via the stimulation of monocytic cells, contribute to the pathogenesis of CF airways disease.
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