179 Attenuation of innate immune response due to a host-adapted cystic fibrosis ST-245-SCC*mec*I-MRSA strain compared with their isogenic ancestor

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an emerging pathogen causing chronic pulmonary infection in cystic fibrosis (CF). We compare the the innate immune response induced by the first MRSA recovered in a CF-patient and the corresponding isogenic strain obtained 13 years after.
Material and Methods: 36 MRSA isolates were obtained from a single CF-patient along 13 years (1996–2009). Genetic diversity was analyzed by PFGE-*Xba*I and MLST. Immunogenic ability was determined in parallel in two isogenic strains corresponding to the first and the last isolates obtained (CF-MRSA-1996 and CF-MRSA-2009) and compared with the HARMONY ST245-MRSA reference strain. Purified human peripheral blood monocytes, isolated from healthy volunteers, were incubated with freshly prepared sonicated crude extracts of both CF-strains for 3 and 6 h. Then, TREM-1, TNF-α, IL-6, IL-12, IL-10 and IRAK-M expression were analyzed at both mRNA and protein levels using real-time quantitative PCR, Flow Cytometry and Western blot.
Results: All MRSA isolates were almost genetically identical and belonged to the ST245-SCC*mec*I-*pvl*$^{-}$. CF-MRSA-2009 provoked 50% less pro-inflammatory response in cultures of human monocytes than the isogenic CF-1996-MRSA isolate and also less than the reference strain. However, the pro-inflammatory response with the reference strain was 1.5 times higher when compared with both CF-MRSA isolates.
Conclusion: Chronic colonization of a single ST247-SCC*mec*I-MRSA clone determines an attenuation process of triggering the human innate immune system. This observation might explain the tolerance of the host towards *Staphylococcus* observed in CF patients.

180 Upregulation of dendritic cells during chronic *Pseudomonas aeruginosa* lung infection in BALB/c mice

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Cystic fibrosis patients often acquire a chronic *Pseudomonas aeruginosa* lung infection. The adaptive immune response is characterised by a Th2 dominated response with a pronounced antibody production. However, patients with a more Th1 dominated response have a better lung function. To further investigate the role of the adaptive immune response we aimed at characterising the dendritic cells (DCs) in a mouse model of chromic *P. aeruginosa* lung infection.
P. aeruginosa (PAO579) were embedded in seaweed alginate beads (0.7×10^9 CFU/ml). Female BALB/c (n = 35) were infected with 0.04 ml of the beads in the left lung. Mice were killed 1, 2, 3, 7 or 10 days after infection. Lungs and regional lymph nodes were collected. DCs were enriched by magnetic cell sorting with anti-CD11c from mechanically degraded lungs and regional lymph nodes. DCs were defined as $CD11c^+$ and $MHCII^+$ $CD45^+$ cells and analysed by flow cytometry. Numbers of DCs were determined using Truecount (R&D) and presented as median log number/ml.
In the lungs a rise in the number of DCs were detected from day 1 [5.49 (5.12–6.14)] to day 2 [6.24 (6.15–6.54), $p \leqslant 0.02$). From day 3 [5.83 (5.62–6.15)] to day 7 [6.38 (6.27–6.47)] a further increase in DCs was observed ($p = 0.02$). However, at day 10 DCs decreased [6.05 (5.67–6.23), $p = 0.05$]. In the regional lymph nodes DCs increased from day 1 [4.64 (4.09–5.2)] to day 3 [5.83 (5.79–6.15), $p < 0.02$]. Lymph DCs remained stable through day 7 [6.52 (5.73–6.54)] but decreased at day 10 [5.15 (5.06–5.26), $p < 0.02$].
In conclusion, the present study shows a significant increase in the DCs during chronic *P. aeruginosa* lung infection, indicating that DCs may be subject to immune modulatory treatment.

181 Neutrophils stain positive for IL-17A in sputum of CF patients

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IL-17A is a neutrophil attracting cytokine that plays a role in the pathogenesis of numerous diseases, including the pathophysiology of lung inflammation in CF patients. IL-17A is produced by different types of T cell populations, such as CD4 positive T helper 17 cells (Th17 cells) and natural killer T (NKT) cells. However, T cells are not the only source of this cytokine. Neutrophils in the broncho-alveolar lavage fluid of severe combined immunodeficiency (SCID) mice are also able to produce IL-17 upon LPS stimulation [1]. Since sputum of CF patients contains numerous neutrophils as well as high IL-17 levels, we hypothesized that these neutrophils could be a main source of IL-17 production.
We collected induced sputum samples of two adult CF patients. Cytospins were performed on lysine-coated glass slides. Unspecific binding sites were priorly blocked and IL-17 was immunodetected on permeabilized cells by immunofluorescence using a monoclonal antibody against human IL-17A and an Alexa-Fluor 488 fluorochrome-conjugated secondary antibody. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI).
Neutrophils in the sputum of both CF patients were positive for IL-17A.
These preliminary data show that IL-17 is produced by neutrophils in the sputum of CF patients, suggesting that IL17 might play a role in maintaining continued presence of neutrophils, responsible of lung tissue destruction in CF.

Reference(s)

[1] Ferretti S, Bonneau O, Dubois GR, Jones CE, Trifilieff A. IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. J Immunol 2003 Feb 15; 170(4): 2106–12.

182 Characterization of sulfamethoxazole metabolite-specific T-cell activation in patients with cystic fibrosis

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Antigen-specific T-cells are involved in the pathogenesis of sulfamethoxazole (SMX) hypersensitivity. The aim of this study was to utilize lymphocytes from SMX hypersensitive patients with and without cystic fibrosis (CF) to explore the chemical basis of drug-specific T-cell activation. Lymphocytes (1.5×10^5; 200 μl) from 6 patients (3 with CF) were stimulated to proliferate when incubated with SMX (100 μg/ml; SI range 2–15) and nitroso SMX (80 μM; SI range 2–28). Over 5000 T-cell clones were generated from the hypersensitive patients and studied in terms of their antigen-specificity. 98 clones were stimulated with SMX (50–200 μg/ml; SI range 2–39, 66% CD4+, 21% CD8+, and 11% dual positive), while 154 clones proliferated in the presence of nitroso SMX (20–160 μM; SI range 2–49, 87% CD4+, 10% CD8+ and 3% dual positive). In patients without CF, SMX stimulated T-cells via a direct interaction with MHC molecules. Nitroso SMX stimulated T-cells by (1) binding irreversibly to MHC and (2) a classical hapten mechanism involving antigen processing. In contrast, T-cell clones from patients with CF were stimulated with both SMX and nitroso SMX. The response to SMX was dependent on metabolism by antigen presenting cells (APC) and intracellular adduct formation. The SMX-specific proliferative response was inhibited by the enzyme inhibitors methimazole and 1-aminobenzotriazole. Furthermore, pulsing APC for 16 h generated an antigenic signal for T-cells. These results demonstrate that intracellular SMX metabolism by immune cells and subsequent protein adduct formation represents an important antigenic signal in hypersensitive patients with CF.