220* IP-10 induction after LPS stimulation is compromised in cystic fibrosis bronchial epithelial cells

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As part of the innate immune system, airway epithelial cells secrete proinflammatory cytokines after activation of Toll-like receptors (TLR) by pathogens. We have shown that in CF bronchial epithelial cells, a reduced surface expression of TLR-4 causes a diminished IL-8 response to LPS. However, there is no information regarding activation of the MyD88 independent TLR-4 signaling pathway by LPS which results in the secretion of the T-cell recruiting chemokine IFN-γ-inducible protein (IP)-10. Therefore, we investigated the induction IP-10 in CF bronchial epithelial cell line CFBE41o- and its CFTR corrected isotype.

CF cell line CFBE41o- (CFTR mutation ΔF508/ΔF508) and its CFTR corrected isotype (wild-type CFTR plasmid transfectant) were cultivated under air-liquid interface conditions. TLR-4 surface expression was revealed by FACS analysis. Basal and LPS stimulated IP-10 secretions were analyzed by ELISA.

TLR-4 surface expression was significantly reduced in CFBE41o- by a factor of 2, compared to the CFTR-corrected cells. CF cells exhibited higher baseline IP-10 secretions compared to the CFTR-corrected cells, but showed no response to LPS. In CFTR-corrected cells, stimulation with LPS increased IP-10 secretions by a factor of 3. Incubating cells with siRNA directed against TLR-4 inhibited the LPS stimulated increase of IP-10 in CFTR-corrected cells. In all experiments, CFBE41o- cells transfected with a control plasmid showed results similar to those observed for the CFBE41o- cells.

The reduced TLR-4 surface expression in CF cells causes the loss of induction of IP-10 by LPS. This could compromise adaptive immune responses in CF due to a reduced T-cell recruitment.

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221* Drug specific T cells in patients with a history of non-immediate hypersensitivity reactions

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Intravenous antibiotics are essential to treat pulmonary exacerbations in cystic fibrosis (CF). Up to 5% of treatment courses are complicated by hypersensitivity reactions, most commonly to beta-lactam antibiotics. They are usually non-immediate and an accelerated response is seen following re-exposure in keeping with an immunological process. Symptoms usually consist of maculopapular rashes, fevers, and arthralgia. The aetiology of these reactions has never been established. 25 CF patients with previous non-immediate reactions to intravenous antibiotics have been assessed to date. These patients were skin prick test negative to the causative antibiotics and had no history suggestive of an IgE mediated reaction. 8 non-allergic CF patients were used as tolerant controls together with 5 non-CF naive controls.

The lymphocyte transformation test (LTT) identifies drug specific T cells in-vitro. During the LTT patient's lymphocytes are cultured with the antibiotic, if drug specific T cells are present proliferative responses are seen. Responses 3 times greater than the negative control readings are considered positive.

Reliable positive responses have been identified in patients with reactions to piperacillin (12/16, 75%), co-trimoxazole (5/9, 56%), and colomycin (6/14, 43%). Importantly, no proliferative responses were seen in the tolerant patients.

It is likely that the non-immediate reactions seen frequently in CF originate from drug specific T cells. Investigations to clone and type these cells, together with evaluating their cytokine profiles, have been commenced.

222* Cytokine production in the differentiating human airway epithelium

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A pro-inflammatory state of the human airway epithelium has been proposed to explain the exaggerated recruitment of neutrophils to the airways of patients with cystic fibrosis (CF). We developed a model of primary Human Airway Epithelial Cell (HAEC) culture isolated from nasal polyps. HAEC could be amplified up to 3 passages, and for each passage differentiated at the air-liquid interface into a mucociliated pseudostratified epithelium within 30 days. The production of IL-8, which is highly elevated in CF HAEC cultures, decreased with time of differentiation, as shown by ELISA. We next studied the release of IL-8 and of IL-6 induced by TNF-alpha in 30 days HAEC cultures and as a function of cell passage. Although CF HAEC showed enhanced basal and stimulated release of IL-8 and IL-6 at early passage, this difference vanished for higher cell passages. Finally, the production of 79 cytokines was analyzed by a cytokine array in 7- and 30-days old cultures generated from passage 2 HAEC. Seven days CF HAEC cultures showed increased production of GM-CSF, GRO-alpha, IL-5, IL-6, IL-7 and MCP-1 but decreased production of TIMP-1 as compared to non-CF cultures. This difference in cytokine production, with the exception of IL-6, disappeared in 30 days HAEC cultures. Thus, our results revealed a huge difference in cytokine production in non-differentiated CF HAEC as compared to non-CF cells; this difference was abolished in well differentiated and polarized HAEC. They also indicate that there is no intrinsic CFTR-dependent anomaly of cytokine secretion by CF HAEC. This data may reconcile opposite reports based on different models of culture and address the question of the role playing by proliferation factors on the innate immune response in CF.

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223 Decreased IL-8 secretion and expression by fluvastatin in primary human macrophages and in the whole blood from adult patients with cystic fibrosis

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Early in life, CF patients become infected with microorganisms including bacteria, particularly *Pseudomonas aeruginosa*, and fungi, Aspergillus fumigatus. Recent research has identified anti-inflammatory properties of statins beside their lipid-lowering effect. Therefore, we have investigated the effect of fluvastatin on IL-8 secretion, using ELISA, and gene expression, using quantitative PCR. Human primary macrophages were obtained by differentiation of peripheral blood mononuclear cells with GM-CSF. Besides whole blood from adult CF patients were collected at the Rennes Teaching Hospital (France) accordingly to the local ethical committee. Whole blood or macrophages were pretreated 1 h by fluvastatin and incubated 24 h with lipopolysaccharide from *Pseudomonas aeruginosa* and/or Aspergillus fumigatus antigens. In both cultures, IL-8 protein levels were dose-dependently increased when cells were stimulated by Aspergillus antigens or lipopolysaccharide. Additive effects were observed in case of co-stimulation. We also demonstrate that fluvastatin strongly decreases protein levels of IL-8 in a concentration-dependent manner. Similarly, in macrophages, fluvastatin induced potent down-regulation of IL-8 mRNA levels.

In conclusion the inhibitory effects of fluvastatin on systemic and local inflammation could reveal important therapeutic potential of statins in various pathological conditions associated with over-production of pro-inflammatory cytokines and chemokines like observed in cystic fibrosis.

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