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, fever, 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** 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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