169 The presence of proteinase 3 at the plasma membrane after apoptosis decreased the rate of phagocytosis by macrophages: a new pro-inflammatory role of membrane proteinase 3 in CF neutrophils

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Neutrophil-dominated airway inflammation observed in CF, results in high concentrations of serine-proteases, especially human neutrophil elastase (HNE) and proteinase 3 (PR3), which are granular proteinases stored in neutrophils, and which are released during inflammation. The aim of this study was to understand the role of serine proteinases in mechanisms of inflammation associated with in CF. We have observed that:

1. PR3 but not elastase, is expressed at the plasma membrane not only during degranulation but also during apoptosis and that this was much pronounced in neutrophils from CF patients and
2. a significant delay of apoptosis in neutrophils from CF patients.

Our working hypothesis was that apoptosis-induced PR3 membrane expression contributes to the impairment of apoptosis observed in neutrophils from CF patients. We investigated whether PR3 could interfere with the mechanisms of clearance of neutrophils. We have performed phagocytosis experiments using control and apoptotic RBL stably transfected either with active PR3 (RBL/PR3) or with an inactive mutant (RBL/PR3S203A), and human monocyte-derived macrophages. We have observed a lesser percentage of phagocytosis with the apoptotic RBL/PR3 thus providing evidence that PR3 expressed at the plasma membrane in apoptotic neutrophils decreased the rate of clearance by macrophages. We concluded that PR3 membrane expression might represent a potential therapeutic target to down-regulate neutrophil-dominated inflammation in CF.

170 Does defective apoptosis play a role in CF lung disease?

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Aims: Although apoptotic dysfunction has recently been suggested in CF, there are few studies reported concerning apoptosis in CF with controversial results. The aim of this study was to investigate apoptosis in CF human lung tissues and compare with non-CF bronchiectatic and healthy lung tissues. We also investigated the relation between apoptosis and histopathological features of tissues and the factors influencing apoptosis.

Methods: Lung tissue samples from CF (n=30), non-CF bronchiectasis (n=28, BE group) and normal control cases (n=24, C group) were included in the study. H&E stained archived slides of tissues were classified and TUNEL method was used to detect DNA fragmentation.

Results: Apoptotic cells were significantly increased in the CF group compared to BE and C groups ($p=0.046$). Bronchopneumonia (BP) findings were positive in 15 CF cases (50%) and 2 BE cases (7%); none of the cases in C group had BP findings ($p=0.0001$). In 25 patients, apoptosis was detected in the lung tissues (14 CF, 5 BE, 6 C cases), while BP findings were present in 17 cases (15 CF, 2 BE cases). Apoptotic findings were significantly increased in cases with BP (n=17) compared to cases without ($p=0.04$).

Conclusions: Excess level of apoptosis might be the result of enhanced occurrence of BP findings because agents causing BP which may also induce apoptosis in alveolar epithelial cells. Apoptotic cells were alveolar epithelial cells in great majority of the patients. They were not detected in other locations where CFTR expression is much more prominent. We might postulate that the increased apoptotic findings in the alveolar epithelium was related with the presence of chronic infections rather than CFTR dysfunction.

171 Investigation of neutrophil apoptosis in Cystic Fibrosis children using a proteomic approach

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Polymorphonuclear neutrophil (PNN) constitutes the therapeutic target for cystic fibrosis (CF) because the sustained lung inflammation in CF is dominated by PNN. Our working hypothesis is that this persistence might be partially explained by a defect in apoptosis thus resulting in PNN accumulation at inflammation sites. Previous results obtained in our laboratory suggest that PNN have a constitutive disorder which can promote inflammation. The aim of the present study is to investigate the mechanisms of apoptosis in PNN from CF patients and to identify proteins modulated during apoptosis in CF. First, we observed a significant delay in the apoptosis of PNN from CF patients as compared with control donors. This delay in PNN apoptosis was observed whatever the pathway of apoptosis induction either physiological or pharmacological with gliotoxin or via death receptors. However, this delay was more important in apoptosis induced by anti-Fas which has been linked with intracellular concentration of glutathione. Second, we showed that under glutathione inhibition by diamide, apoptosis of PNN from control donors and CF patients were enhanced and the delay in the apoptosis of PNN from CF patients was not significant any more. Finally, we are currently investigating whether cytosolic proteins could be involved in the regulation of apoptosis in CF patients by using proteomic approaches. The analysis of 2D gels indicates several cytosolic proteins differentially expressed between apoptosis and basal conditions. Most interestingly, we also have observed some significant differences between controls and CF patients.

172* The Quorum Sensing system controls the secretion of a cytotoxic activity by *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is the predominant micro organism of chronic lung infections in cystic fibrosis (CF) patients. *P. aeruginosa* colonizes and infects the lungs by forming biofilm microcolonies. The alginate content of the biofilm is providing protection against the immune defence and antibiotic treatment. In addition, we have previously demonstrated that the Quorum Sensing system (QS) is also contributing to the tolerance of the biofilm growing bacteria towards antibiotics and the bactericidal activity of the polymorphonuclear leukocytes (PMNs).

To further explain the protective role of QS against the immune system we evaluated the effect of QS on the release of cytotoxicity by incubating human PMNs in sterile filtered supernatants from *P. aeruginosa* cultures. Flow cytometry showed a rapid disintegration of PMNs incubated with supernatants from QS-competent PAO1 biofilm. No cytotoxic effect was found in supernatants from the QS-incompetent double-knockout strains (Δ*lasI rhlI* and Δ*lasR rhlR*). In addition, when Δ*lasI rhlI* was grown in the presence of acyl homoserine lactones (C4 and C12) the supernatant became cytotoxic. Fluorescence time lap microscopy showed a fast release of DNA from the PMNs during the QS induced necrosis. As the supernatant from PAO1 was also hemolytic, the plasma membrane is likely to be a target of the cytotoxic activity. The cytotoxic effect is unlikely to be due to the QS-signal molecules *per se*, since supernatants from Δ*lasR rhlR* grown in the presence of C4 and C12 did not become cytotoxic.

In conclusion, the QS of *P. aeruginosa* is inducing an activity that rapidly kills the PMNs. This may help the bacteria to escape the immune system and to promote colonization and infection of the CF lungs.