Acute inhibition of the cystic fibrosis transmembrane conductance regulator leads to inflammation: evidence for a functional association with Annexin A1

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Cystic fibrosis (CF) is a monogenic disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. It is characterized by chronic bacterial lung infections and inflammation. Having previously shown de-regulation in Annexin A1 (AnxA1) levels, a glucocorticoid inducible protein with strong anti-inflammatory properties, in both human primary CF epithelial cells and CFTR-/- animals [1], we set out to determine if this deregulation is at least in part responsible for the aggravated inflammatory profile in this condition.

A pharmacological of the CFTR inhibitor ($CFTR_{172}$; Sigma) was given i.p. (50µg/mouse) with or without zymosan A to C57Black6 (WT) or AnxA1-/- mice and assessing the recruitment of Gr1+ cells at the 4 h time-point, using flow cytometry analysis. AnxA1 expression in peritoneal cells and lavage fluids was determined by Western blotting. Experiments with human neutrophils (PMN) were also conducted adding $CFTR_{172}$ (50µM) prior to LPS (10ng/ml) stimulation: AnxA1 protein and mRNA levels were then measured by a combination of real time PCR, Western blotting and FACS analyses. Data are mean ± SEM of 6 mice or groups.

$CFTR_{172}$ (50µg/mouse) given i.p. -24 and -1h before zymosan potentiated the Gr1+ cell recruitment promoted by 100µg zymosan, from 2.5±0.3 to 7.1±0.4 (n=6, $P<0.05$) cells per animal. an effect not observed in AnxA1-/- mice. Cytokine array revealed discrete changes upon $CFTR_{172}$ administration, particularly evident for IL-6 where levels mirrored cell recruitment, i.e. higher after $CFTR_{172}$ in WT mice; augmented in AnxA1-/- mice and not further increased by application of $CFTR_{172}$. Finally, administration of hrAnxA1 to $CFTR_{172}$/zymosan treated WT mice provoked significant reduction (50%, n=6, $P<0.05$) in Gr1+ cell recruitment. *In vitro* assays on human PMN incubated with $CFTR_{172}$ (± LPS) also confirmed AnxA1 deregulations (~50% less cytosolic content, >100% increase in supernatants) at both protein and gene level.

These findings corroborate and extend previous associations between CFTR abnormalities and AnxA1 protein disposition, linking it particularly to the inflammatory process that characterises CF. We have described an experimental model that could help in the identification of novel anti-inflammatory strategies for CF, providing an exciting and novel pharmacological tool in the quest to treat CF.

[1] Bensalem N *et al* MOL CELL PROTEOMICS. 4: 1591-1601, 2005.