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Commentary

Not All Is CFTR – Neutrophils and Cholesterol in Cystic Fibrosis

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Cystic fibrosis (CF) is a death-causing syndrome caused by mutations in the CF *transmembrane conductance regulator* (*CFTR*) gene. The most frequent mutation, occurring in 70% of all CF chromosomes, is the *F508del*, causing a defect both in the processing and the function of the protein. Other mutations are less represented, such as the *G551D* mutation which accounts for 4%. The lungs of CF individuals are affected by opportunistic bacterial infections and an ensuing chronic inflammatory response, the hallmark of which is represented by a high burden of pro-inflammatory mediators and influx of neutrophils (Gifford and Chalmers, 2014). Continuous production and secretion of neutrophil products, such as proteases, is responsible for degradation of extracellular structures in the airways, producing bronchiectasis and end stage lung disease (Sly et al., 2013). Lung transplantation is the only therapeutic measure which can be curative for these patients with respiratory failure (Adler et al., 2009). In recent times however, novel small molecules that target the underlying basic defect in CFTR have been advanced to the point where clinical application has become a reality (Quon and Rowe, 2016). Ivacaftor is a potentiator of CFTR activity as a chloride channel and was approved for the treatment of patients with CF aged 6 and older with the *G551D*-CFTR mutation, demonstrating clinical efficacy at the level of sweat chloride and respiratory symptoms and correlating with a reduction in the bacterial lung burden (Rowe et al., 2014). A number of studies on the effect of ivacaftor at the level of neutrophil function are available (Pohl et al., 2014; Bratcher et al., 2016; Guerra et al., 2017).

In *EBioMedicine*, White et al. (2017) studied plasma membrane proteins and cholesterol composition in CF *F508del* (CFΔ) neutrophils in comparison with healthy control neutrophils. They explored whether chronic inflammation is responsible for altered plasma membrane structure leading to increased neutrophil adhesion and transmigration, or whether these changes were an intrinsic CFTR-related phenomenon. CFΔ neutrophil plasma membranes and lipid rafts were enriched in the adhesion integrin CD11b, while showing reduced cholesterol levels. To determine if these alterations were due to an endoplasmic reticulum (ER) stress response, they demonstrated that ER stress associated proteins GRP78 and ATF6 were increased in CFΔ neutrophils, accompanied by an increase in Ca^{2+} and decrease in caveolin-1 cytosolic levels. Interestingly, these alterations were not detected by pharmacological inhibition of CFTR function. Since caveolin-1 has been implicated in cholesterol transfer to the plasma membrane, the reason for lower levels of caveolin-1 in CFΔ neutrophils was investigated. In vitro and in vivo results confirmed the cysteine protease μ-calpain was responsible for the degradation of caveolin-1. Pro-inflammatory mediators (CXCL8, CXCL8 and TNF-α) abundant in CFΔ plasma were found subsequently to cause increased expression of GRP78 protein, Ca^{2+} cytosolic levels, and μ-calpain activity, and reduced caveolin-1 expression in HL60-derived neutrophils. Findings in post-transplant CF *F508del* patients revealed that the altered cholesterol composition (and linked alterations in Ca^{2+} and μ-calpain levels) of circulating neutrophils is associated with chronic inflammation leaking into the systemic compartment. On the other hand, ivacaftor therapy reduced cytokine levels in plasma of heterozygous *G551D*/*F508del* treated patients, along with reduced ER stress, increased cholesterol content, and reduced adhesion of neutrophils.

From this insightful study, a chain of events emerges. Chronic pulmonary inflammation, leaking into the circulation, leads to alterations in neutrophils, characterized by ER stress-dependent increased Ca^{2+} cytosolic levels and μ-calpain activity, resulting in caveolin-1 proteolytic cleavage, reduced membrane and lipid raft cholesterol content, and increased cell adherence.

This study adds a novel player in the complex pathomechanism of CF-associated lung inflammation, i.e. cholesterol. Several studies have found CFTR-dependent altered functions in neutrophils and mononuclear cells, although with varied results observed depending on the ex-vivo and in vivo-animal models, including alterations in lipidic profiles (Bruscia and Bonfield, 2016). White et al. focussed on cholesterol, an important molecule in determining membrane fluidity and lipid raft composition which mechanistically increase neutrophil adherence. From their results, a picture that the progression of CF lung disease is due to chronic inflammation, in addition to the CFTR defect (Pohl et al., 2014), is emerging. This hypothesis may be confirmed with patients bearing disease-causing mutations other than *F508del* and *G551D*. However, the data in this study for lung transplantation and ivacaftor therapy suggest that bacterial burden and inflammation may play a significant role in modifying the pro-inflammatory functions of neutrophils. Further studies could evaluate young patients to determine if this mechanism is already in action at the onset of CF lung disease and a comparison between chronic and acute exacerbation states might reveal if these alterations could contribute to the discovery of biomarkers specific to CF lung disease.

Disclosure

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