Oxidative stress in cystic fibrosis lung disease: an early event, but worth targeting?

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A recent study showed that oxidative stress occurs early in CF lung disease and represents a potential drug target http://ow.ly/vdH2P

Oxidative stress is a universal biological response, which plays a major role in a variety of inflammatory disease conditions, such as cardiovascular diseases or sepsis. In the lung, harmful oxidative stress mainly occurs in nonresolving pathologies, such as cystic fibrosis (CF) lung disease [1, 2] or, to a lesser extent, chronic obstructive pulmonary disease [3, 4]. In CF, neutrophils are continuously recruited to the airways and liberate their toxic ingredients, particularly proteases and oxidants, in an uncontrolled fashion [5]. Anti-proteases and anti-oxidants shield the lung from free proteolytic and oxidative damage [6]. However, in CF the amount and duration of neutrophilic inflammation overwhelms these defence systems [2, 7]. The released proteases and reactive oxygen species, physiologically the neutrophil's major armamentarium to kill intracellular pathogens, as evidenced in patients with chronic granulomatous disease [8], degrade extracellular matrix components and immune receptors and oxidise proteins in the CF airway microenvironment in an uncontrolled manner.

Glutathione is the major antioxidant shield in the epithelial lining fluid of the human lung and protects this vulnerable area from oxidative stress. Previous reports supported the concept that the cystic fibrosis transmembrane conductance regulator (CFTR) itself transports glutathione, providing a potential explanation for the low glutathione levels in CF airways [9–11]. Alternatively to this CFTR-linked mechanism, activated neutrophils are also capable of oxidising and thereby disabling glutathione [12]. To date, the relative contribution of these two mechanisms and the time-point when glutathione loss occurs in CF lung disease *in vivo* remains poorly understood.

In this issue of the *European Respiratory Journal*, Kettle *et al.* [13] take a closer look at the relationship between glutathione and neutrophilic inflammation by studying bronchoalveolar lavage fluid from 167 infants with CF with a mean age of 3 years. Kettle *et al.* [13] studied a whole panel of glutathione oxidation products and neutrophil-derived enzymes that can oxidise glutathione. This study provides evidence that: 1) loss of glutathione occurs early in CF lung disease; 2) oxidised glutathione closely correlates with neutrophil-derived products, mainly hypochlorous acid; and 3) pulmonary infection enhances oxidative stress and oxidative loss of glutathione.

The question arises of how specific are these findings for CF and what are the therapeutic consequences? We know that glutathione levels in airway lining fluids are low in a number of inflammatory conditions beyond CF, including severe asthma [14]. In previous studies, glutathione levels were found to be decreased in

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patients with advanced CF lung disease [7], whereas in younger CF children only a tendency towards lower levels was found in infected CF patients [15]. When viewed in combination, this might explain the overlap in glutathione levels in the study by KETTLE *et al.* [13] between CF patients and the control group, consisting of children with persistent respiratory symptoms. Although it remains premature to conclude that neutrophil-derived products are solely responsible for the loss of glutathione in CF airways, the close correlation in this young CF population strongly supports the view that impaired CFTR-dependent glutathione transport is not primarily responsible for the loss of functional (non-oxidised) glutathione in CF airway fluids *in vivo*, but rather its consumption by inflammatory processes.

Therapeutically, augmenting epithelial lining fluid glutathione in CF patients by means of inhalation [2, 16] or oral administration of the glutathione prodrug *N*-acetylcysteine [17] is not a novel approach *per se*, but has not been clinically successful to date [18–20]. Several attempts to deliver glutathione into CF airways were capable of delivering substantial amounts of glutathione [18, 19, 21] and demonstrated reduced superoxide anion formation by inflammatory cells [21], as well as modulation of pulmonary immune responses [22]. However, parameters of oxidative stress (myeloperoxidase, ascorbic acid, uric acid and others) were not significantly affected in these studies [18, 19, 22]. Clinically, these previous studies further failed to show a substantial effect on lung function parameters, as demonstrated recently by a 6-month glutathione inhalation study in adult CF patients [19]. Having observed that, in line with the recent findings from the AREST (Australian Respiratory Early Surveillance Team) study, one may conclude that the events leading to glutathione loss occur very early in CF lung disease and, consequently, have to be targeted as soon as possible in the course of disease in order to prevent irreversible oxidative damage of lung tissue components. Since neutrophils release both oxidants and proteases, which act in a synergistic manner to cause harm to the pulmonary tissue [5], advanced therapeutic approaches may consider inhibiting oxidants and proteases simultaneously and very early in CF lung disease. Targeting proteases isn't a real "success story" in CF lung disease yet. On the one hand, anti-proteases delivered into the airways of CF patients dampened airway inflammation and decreased proteolytic activities [23–25]. However, on the other hand, these studies failed to demonstrate an effect on lung function, probably due to insufficient drug concentrations achieved at the pulmonary site of inflammation, the duration of the studies and/or the fact that those studies were performed in adult CF patients, where proteolytic lung damage may have gone too far to stop it. The recent study by the AREST consortium, in line with their previous study on elastase [26, 27], strongly encourage studies using anti-oxidants and/or protease inhibitors in CF infants instead of adults, even though these studies will require high doses of anti-proteases/anti-oxidants and some time in order to show effects on lung function parameters. As forced expiratory volume in 1 s is not a feasible read-out for children <5 years of age, the multiple-breath washout/lung clearance index method might be used in these studies [28–30]. Glutathione is rapidly oxidised in contact with activated neutrophils, which is a highly relevant mechanism in CF airways, mainly through the action of myeloperoxidase-derived hypochlorous acid [12]. Based on this notion, direct inhibition of neutrophil-derived products that oxidise glutathione, such as hypochlorous acid or myeloperoxidase, could represent a more promising and efficient strategy. This is also discussed by KETTLE *et al.* [13]. Patients with congenital myeloperoxidase deficiency have an absent or very mild clinical phenotype [31], suggesting that these approaches could be well tolerated *in vivo*.

The road is paved with more than one rationale to therapeutically target neutrophil products in early CF lung disease, in order to prevent their harmful and irreversible effect on lung tissue components. The clinical implementation of these concepts is hampered by costs, drug delivery issues and optimised read-outs in this young age group, but the promises make it worth facing these challenges.

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