

# Oxidant stress in asthma

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Asthma is a chronic inflammatory disease of the respiratory tract of unknown aetiology. In severe asthma airway diathesis is profound, and thus apparent. Recently, however, evidence has indicated that specific inflammatory abnormalities exist even in the airways of subjects with mild disease. As inflammation is often associated with an increased generation of reactive oxygen species (ROS), and the biochemical environment in the asthmatic airways is favourable for free radical mediated reactions, it is rational to surmise that an oxidant stress could be mechanistically important in asthma.

## Cellular and biochemical sources of ROS in asthma

The inflammatory cells recruited to the asthmatic airways have an exceptional capability for producing ROS. Activated eosinophils, neutrophils, monocytes, and macrophages can generate superoxide ( $O_2^-$ ) via the membrane associated NADPH-dependent complex. Subsequently, dismutation of  $O_2^-$  gives hydrogen peroxide ( $H_2O_2$ ).  $O_2^-$  and  $H_2O_2$  per se are moderate oxidants; however, both species are critical for the formation of potent cytotoxic radicals in biological systems through their interaction with other molecules. For example, hydroxyl radical ( $OH^\cdot$ ), a powerful and indiscriminate oxidant, can be produced from  $O_2^-$  and hypohalous acids (HOCl or HOBr). The latter component can be formed from  $H_2O_2$  and a halide ( $Cl^-$  or  $Br^-$ ) in a reaction catalysed by myeloperoxidase (MPO) provided by neutrophils and monocytes,<sup>1</sup> or eosinophil peroxidase (EPO) from eosinophils.<sup>2</sup> MPO preferably utilises  $Cl^-$  as a halide, whereas EPO uniquely prefers  $Br^-$ . Moreover, a recent study has shown that MPO and EPO can use nitrite (a major end product of nitric oxide (NO) metabolism) and  $H_2O_2$  as substrates to promote formation of reactive nitrating intermediates.<sup>3</sup> The oxidative injury caused by eosinophils can be substantial because the cells possess several times greater capacity to generate  $O_2^-$  and  $H_2O_2$  than neutrophils,<sup>4</sup> and the content of EPO in eosinophils is 2–4 times higher than the amount of MPO in neutrophils.<sup>5</sup> Another example of a powerful oxidant and a nitrating radical is peroxynitrite (ONOO $^-$ ) produced from the reaction of  $O_2^-$  with NO.<sup>6</sup> This pathway may be involved in asthma because the concentration of NO is increased in the asthmatic airways.<sup>7</sup> In addition to the recruited inflammatory cells, the constitutive airway cells such as epithelial cells are also potential sources of ROS.<sup>8</sup> Moreover, cells recovered from bronchoalveolar lavage (BAL) fluid and blood of asthmatic subjects have been shown to generate greater amounts of ROS at

baseline and after stimulation ex vivo than in normal subjects, a feature which in some studies correlated with disease severity. This suggests that the biochemical milieu in asthma contains factors which prime oxidative pathways in vivo.<sup>9–14</sup>

As in many other pathological conditions, the oxidant “burst” in asthma is presumably a self propagating non-specific process initiated by the concurrent action of numerous inflammatory pathways. Several asthma mediators including lipid mediators,<sup>15</sup> chemokines,<sup>16, 17</sup> adhesion molecules,<sup>18</sup> and eosinophil granule proteins<sup>19</sup> are potential stimuli or promoters of ROS production. In addition to endogenous sources, some environmental factors linked to asthma such as air pollutants (for example, ozone, diesel exhaust particles) may cause an extreme increase of ROS generation in the airways.<sup>20</sup>

In principle an increase in the production of ROS is problematic because oxidation of proteins, DNA, and lipids may cause direct tissue injury or evoke a variety of cellular responses through the generation of secondary reactive species.<sup>21</sup>

## Antioxidants and asthma

Numerous disturbances of antioxidant defence mechanisms have been described in asthma. For example, the expression of the asthma phenotype has been linked to reduced selenium status, an essential element for the normal activity of glutathione peroxidase,<sup>22, 23</sup> and insufficient dietary intake of vitamins with antioxidant properties, particularly in smokers.<sup>24–26</sup> Decreased activity of copper and zinc containing superoxide dismutase (Cu,Zn-SOD) in bronchial epithelial cells and BAL fluid cells has recently been found in asthmatic subjects not using inhaled corticosteroids compared with corticosteroid treated asthmatic patients and normal subjects. This was caused by an altered expression of the enzyme, which was similar in all groups. Treatment with inhaled corticosteroids abolished the abnormality. No difference in the activity of manganese containing SOD (Mn-SOD), catalase, and glutathione peroxidase was detected between asthmatic and control subjects. The activity of SOD correlated with non-specific airway reactivity assessed by methacholine challenge.<sup>27, 28</sup> A polymorphism in antioxidant enzymes—for example, Mn-SOD and glutathione S-transferase—has also been reported in asthmatic subjects.<sup>29</sup> Nevertheless, the significance of these abnormalities in the pathogenesis of asthma is unclear at this time.

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### Could increased activity of ROS have a role in the pathogenesis of asthma?

The exposure of animals in vivo and human airway tissue in vitro to different oxidants has been shown to produce airway constriction and hyperresponsiveness.<sup>30-32</sup> However, the relevance of these experiments to human asthma is uncertain. Of interest, however, are recent studies suggesting that ROS mediated reactions may alter or induce some inflammatory and immunological cellular responses—for example, through the generation of second messengers. It is important to emphasise that the consequences of oxidant stress may vary in normal lungs and in lungs with pre-existing allergic inflammation caused by a different biochemical and cellular environment. Nevertheless, some of the effects mediated by ROS with a feasible role in asthmatic inflammation and airway remodelling include activation of transcription factors such as STATs and NF- $\kappa$ B,<sup>33-35</sup> modulation of glucocorticoid dependent signal transduction,<sup>36</sup> stimulation of phospholipase and eicosanoid synthesis,<sup>37-39</sup> induction of growth factors,<sup>40</sup> cytokines,<sup>41</sup> and modification of cellular ion transport mechanisms.<sup>42</sup> Specific studies will be necessary to establish which of these effects could be involved in asthma.

### Evidence for oxidative stress in asthma

Much of the evidence for the activity of ROS in asthma is indirect or circumstantial because there are no reliable methods to assess oxidative stress in vivo. Thus, the measurement of increases in NO, H<sub>2</sub>O<sub>2</sub>, and pentane in exhaled gas or breath condensates, analysis of lipid peroxidation based on diene conjugate and thiobarbituric acid (TBA), and the assessment of substrate oxidisability (or spin trapping) of free radical adducts ex vivo all have low sensitivity and specificity. However, using these procedures, an augmented production of ROS has been found in adults and children with distinct asthma severity and acute exacerbations of asthma.<sup>12 43-48</sup> The specific measurement of F<sub>2</sub>-isoprostanes (F<sub>2</sub>-IsoPs), stable prostaglandin-like arachidonate products formed on membrane phospholipids by the action of ROS, has recently been found to be a sensitive and reliable non-invasive method for assessing oxidant stress in vivo. Indeed, F<sub>2</sub>-IsoPs are increased in a number of human vascular and inflammatory disorders in which oxidant stress has been thought to play a significant role.<sup>49</sup> Using a sensitive and specific mass spectrometry method we found increased concentrations of urinary F<sub>2</sub>-IsoPs in patients with atopic asthma following inhaled allergen challenge. The formation of F<sub>2</sub>-IsoPs was a specific response to allergen because the non-specific bronchoconstrictor methacholine did not cause an increase in F<sub>2</sub>-IsoPs. The measured compounds were non-cyclooxygenase products because they were not abrogated by pretreatment of the subjects with adequate doses of either aspirin or indomethacin. F<sub>2</sub>-IsoPs were also increased in the BAL fluid 24 hours after segmental instillation of the allergen and the increase was inhibited

by pretreatment with inhaled corticosteroids, which suggests that steroids may act in part by restraining oxidant stress. Thus, this study provided direct evidence that oxidant injury occurs in allergic inflammation.<sup>50</sup> This conclusion was further validated by showing that inhaled allergen challenge caused enhanced excretion of 2,3-dinor-5,6-dihydro-15-F<sub>2t</sub>-IsoP (F<sub>2t</sub>-IsoP-M), the major urinary metabolite of 15-F<sub>2t</sub>-IsoP (8-iso-PGF<sub>2 $\alpha$</sub> ).<sup>51</sup> Measurement of a metabolite of F<sub>2</sub>-IsoPs provides a more reliable index of total systemic production of IsoPs because, unlike unmetabolised F<sub>2</sub>-IsoPs, the metabolite cannot be produced in the kidney nor be generated artefactually by auto-oxidation of arachidonic acid during sample handling and storage (Dworski *et al.* unpublished data). We have also found increased release of F<sub>2</sub>-IsoPs and F<sub>2t</sub>-IsoP-M into the urine of patients with aspirin induced asthma following challenge with inhaled lysine-aspirin (Dworski *et al.*, unpublished observation).

If ROS are important in asthma, enhancement of the antioxidant defences would be expected to have beneficial effects in the disease. In this regard the available data in humans are unimpressive. Unfortunately, one of the major weaknesses of the studies to date is the lack of evidence that the chosen doses and mixtures of antioxidants were effective in vivo.<sup>52 53</sup> We anticipate that measurement of the urinary excretion of F<sub>2</sub>-IsoP-M may provide a novel method to define the most effective dosages and combinations of antioxidants to suppress oxidant stress in patients with asthma, which will permit a reliable assessment of the effect of antioxidant treatment on the pathophysiology of the disease process.

### Conclusion

There is evidence that oxidant stress occurs in asthma, which is not surprising considering the inflammatory nature of the disease. However, little is known about the role of ROS in the inflammatory and immunological cascade characteristic of asthma. This is a provocative question because some of the mechanisms may bypass the therapeutic effects of anti-inflammatory drugs, and antioxidant agents could prove useful adjuvant treatment for asthma.

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