Oxidative stress as an initiator of cytokine release and cell damage

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The respiratory system is one of the primary interfaces with the external environment and faces unique demands to handle and detoxify inhaled antigens and particles. It must both control and express inflammatory pathways in ways that preserve the primary functions of the respiratory system while protecting it from invasion by foreign infective agents or antigens. Inhaled air, even under relatively pristine conditions, contains large numbers of particles of diverse origin. Pollens, animal and plant proteins, inorganic dusts, and combustion materials from industry, transportation and smoking create particulate counts in inhaled air ranging from thousands to even millions of particles per cm^3 when ultrafine particles are considered.

The challenge to the lung is to process the vast majority of inhaled antigens without inappropriate and potentially damaging inflammatory amplification. Many components of the immune system involving the lung have long been known to have a blunted or subdued response in comparison with the systemic immune system. This results in the vast majority of inhaled antigenic particles being processed and cleared by the respiratory system without activating the immune system. Alveolar macrophages are generally recognised as relatively poor antigen-presenting cells. This blunted immune response in the lung is critical in order to maintain normal lung function and avoid unnecessary chronic inflammatory reactions to inhaled antigen, while at the same time maintaining an immune system ready to ward off a significant invasion by pathogens.

It is speculated that the redox environment in lung lining fluids is one of the important factors in determining the relative reactivity of responsiveness of the lungs' innate and adaptive immune systems. In the normal lung an extremely high level of extracellular antioxidants functions to maintain extracellular spaces in a highly reduced state and facilitates the maintenance of a blunted immune response [1, 2] (fig. 1).

Airway redox balance in health and disease

In the normal lung the balance between antioxidants and oxidants is sufficient to keep the airway lining fluids and extracellular spaces in a highly reduced state and maintain normal physiological functions. Increases in oxidants or decreases in antioxidants can disrupt this balance. When such an imbalance occurs, is is referred to as oxidative stress and can be associated with diverse lung pathologies including asthma, chronic obstructive pulmonary disease and interstitial lung diseases [3] (fig. 2).

Extracellular superoxide dismutase (ECSOD) is the primary extracellular antioxidant enzyme in the lung and is most highly expressed in airway and vascular walls [4–6]. ECSOD contains an 18 amino acid carboxyterminus region that

Fig. 2. – Extracellular airway oxidants and antioxidants in asthma. Normal airways are replete with antioxidants, such as glutathione (GSH), urate, ascorbate and extracellular superoxide dismutase (ECSOD). In a normal airway, concentrations of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are suppressed by antioxidants and are highly localised, most likely primarily carrying out signalling functions. For instance, neuronal nitric oxide synthase (nNOS) makes nitric oxide (NO) at nerve terminals to dilate airway smooth muscle and constitutive NOS (ecNOS) has functions such as dilating blood vessels. In asthma there are increased eosinophils (EOS) and polymorphonuclear neutrophils (PMN). The airways' inflammation is associated with increases in inducible NOS (iNOS) and membrane oxidases that make superoxide (O_2^-). O_2^- and NO rapidly combine to form peroxynitrate ($ONOO^-$), leading to tissue injury and proinflammatory responses. Increases in ROS/RNS also deplete antioxidants. For example, GSH is oxidised to GSSG. The role of ECSOD is not known, and changes in airway ECSOD levels as either a primary or secondary response could be a factor in perpetuating the asthmatic inflammatory response. GSNO: S-nitrosoglutathione; H_2O_2: hydrogen peroxide; GPx: glutathione peroxidase.
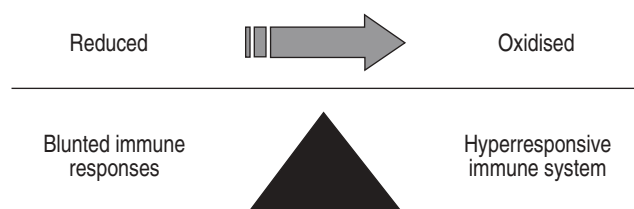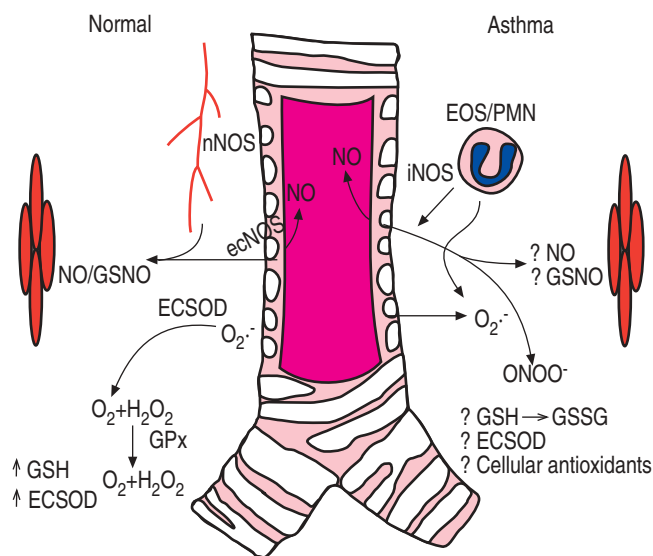

Fig. 1. – Immune system activation *in vivo* is regulated by oxidation and reduction reactions and thus the responsiveness of the immune system is influenced by the general tissue redox state.

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contains six positively charged amino acids. This positively charged region causes ECSOD to bind to heparin and other similar negatively charged molecules in the lung extracellular spaces (fig. 3).
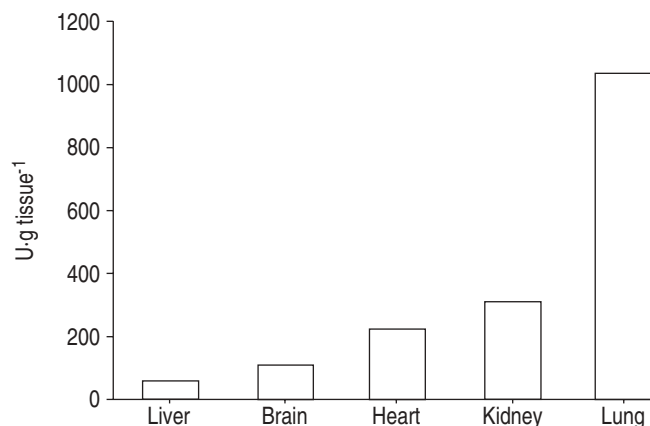

Fig. 3. – Activity of extracellular superoxide dismutase in various tissues showing the unique expression in lung.

Role of changes in airway redox environment on the innate and adaptive immune system

In asthmatic airways the delicate balance between reactive oxygen (ROS)/reactive nitrogen species (RNS) and antioxidants has been shown to be in disequilibrium due to an excess production of ROS/RNS [7]. It is also possible that disequilibrium is related to depletion of antioxidants. The impact is to create a more oxidising environment throughout the airway lining fluids and extracellular spaces. Figure 4 illustrates the hypothesised impact of this enhanced oxidising environment on both the innate and the adaptive immune responses [8]. The enhanced oxidising environment can facilitate the binding of pathogens or antigens to effector cells leading to a hyperresponsive innate immune system. Previous work has shown that an oxidising environment leads to enhanced release of superoxide and nitric oxide, activation and translocation of nuclear transcription factor-κB and enhanced production of cytokines, including tumour necrosis factor-α, interleukin (IL)-1β and IL-12. Similarly, the creation of a markedly reduced environment by addition of antioxidants blunts all of the above primary responses of the innate immune system.

Development of pharmaceutical mimetics of extracellular superoxide dismutase

A number of metal complexes have been described as superoxide dismutase (SOD) mimetics. AEOL 10113 and AEOL 10150 are positively charged, and the positive charges on the four pyridyl or imidizol groups in these compounds give them distribution and function profiles that mimic ECSOD. These compounds are not exact mimetics of ECSOD since there will be some differences in distribution and, as shown in figure 5 and table 1, they have broader antioxidant profiles than ECSOD, which only scavenges superoxide [9].

Thus, AEOL 10113 and AEOL 10150 possess a broad range of antioxidant activities including mimicking SOD and catalase, and scavenging both lipid peroxides and peroxynitrite. These antioxidant mimetics are positively charged and
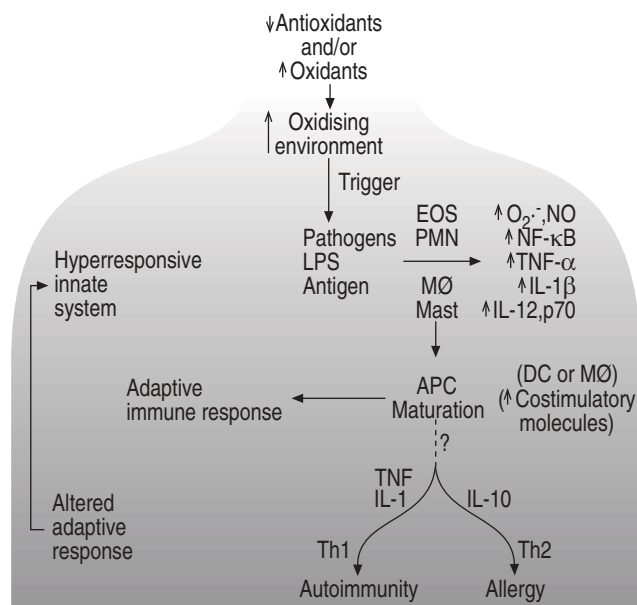

Fig. 4. – Effects of an oxidising environment on the innate and adaptive immune responses. Changing the redox environment in which the immune system operates towards an increased oxidising environment has been shown to lead to a hyperresponsive innate immune system and to the enhanced activation of the adaptive immune responses involving antigen-presenting cell (APC) maturation and T-cell activation. These impacts of the oxidising environment have been demonstrated for autoimmune pathways in models of diabetes and are likely to be similar for the T-helper cell (Th) type-2 pathway, playing a prominent role in the pathogenesis of the asthmatic reaction. EOS: eosinophil; PMN: polymorphonuclear neutrophils; MØ: macrophage; Mast: mast cell; LPS: lipopolysaccharide; $O_2^{.-}$: superoxide; NO: nitric oxide; NF: nuclear transcription factor; TNF: tumour necrosis factor; IL: interleukin; DC: dendritic cells.
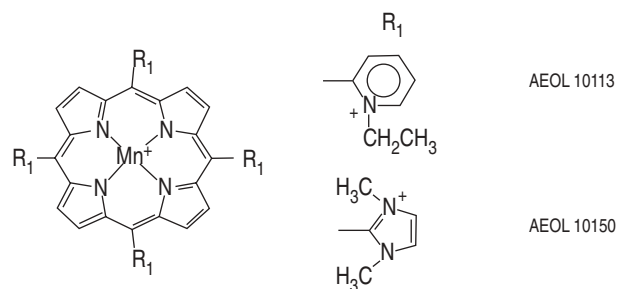

Fig. 5. – Structure of two mangano porphyrins that are effective antioxidants in animal models.

Table 1. – Comparison of antioxidant activities

Antioxidants	SOD U·mg^{-1}	Catalase % activity	LP IC$_{50}$ μM	RP E$_{1/2}$ V
CuZn SOD	5100		15	+0.35
AEOL 10113	8000	0.9	1.0	+0.22
AEOL 10150	14789	0.9	0.5	+0.90

SOD: superoxide dismutase; LP: lipid peroxidation; IC50: inhibitory concentration of 50%; RP: redox potential.

function like ECSOD mimetics and have been shown to be effective in a number of animal models of lung disease [10–14].

An extracellular superoxide dismutase mimetic reduces airway inflammation in a mouse model of asthma

The impact of an ECSOD mimetic (AEOL 10113) on the regulation of allergen-induced airway inflammation and reactivity was evaluated by treating ovalbumin (OVA)-immunised and challenged mice [13]. BALB/c mice were given an intraperitoneal injection of 10 μg OVA and 1 mg alum in 100 μL of phosphate-buffered saline on day 1 and day 14. They were subjected to a 30-min aerosol challenge of either distilled water (dH$_2$O) or 1% OVA in dH$_2$O on days 28, 29 and 30. One-half of the mice in both the dH$_2$O- or OVA-challenged groups were treated with AEOL 10113. The antioxidant treatment was administered by intratracheal instillation at a dose of 2 μg·lung^{-1} and delivered in a volume of 50 μL, 1 h after the OVA challenge on days 28 and 30. Mice were anaesthetised for bronchoalveolar lavage (BAL) 48 h after the last antigen challenge to assess the severity of airway inflammation (fig. 6).

Inhibition of tobacco smoke-induced lung inflammation by a catalytic antioxidant

Cigarette smokers experience airway inflammation and epithelial damage, the mechanisms of which are unknown. One potential cause may be free radicals either in tobacco smoke or produced during persistent inflammation. Inflammation may also be a driving force to cause airway epithelium to undergo changes leading to squamous cell metaplasia. To test whether tobacco smoke-induced inflammation could be reduced by a catalytic antioxidant, AEOL 10150 was given by intratracheal instillation to rats exposed to filtered air or tobacco smoke for 2 days or 8 weeks (6 h·day^{-1}, 3 days·week^{-1}). AEOL 10150 significantly decreased BAL cell number in tobacco smoke-treated rats. Significant reductions in neutrophils were noted at 2 days and macrophages at 8 weeks. Lymphocytes were significantly reduced by AEOL 10150 at both time points. Squamous cell metaplasia following 8 weeks of tobacco smoke exposure was 12% of

the total airway epithelial area in animals exposed to tobacco smoke without AEOL 10150, compared with 2% in animals exposed to tobacco smoke, but treated with AEOL 10150 (p<0.05). These findings show that a synthetic catalytic antioxidant will markedly reduce the adverse effects of exposure to tobacco smoke.

Summary

This article characterised the role of oxidative stress in mediating pathological reactions in the lung and the unique antioxidant defences that the lung possesses. The impact of redox balance in regulating inflammatory and immune reactions were discussed, and the impact of enhancing lung antioxidant capacity in animal models of asthma and chronic obstructive pulmonary disease were characterised.

Fig. 6.–Treatment of mice with AEOL 10113 during ovalbumin (OVA) aerosol challenges inhibits OVA-induced airway eosinophils. BAL: bronchoalveolar lavage. ■: saline/saline; ▪: saline/AEOL 10113; □: OVA/saline; ▨: OVA/AEOL 10113. *: compared with saline/saline; #: compared with OVA/saline. Reproduced from [13] with permission.

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14. Chang LY, Subramaniam M, Yoder BA, et al. A catalytic antioxidant prevents alveolar structural remodeling in bronchopulmonary dyspl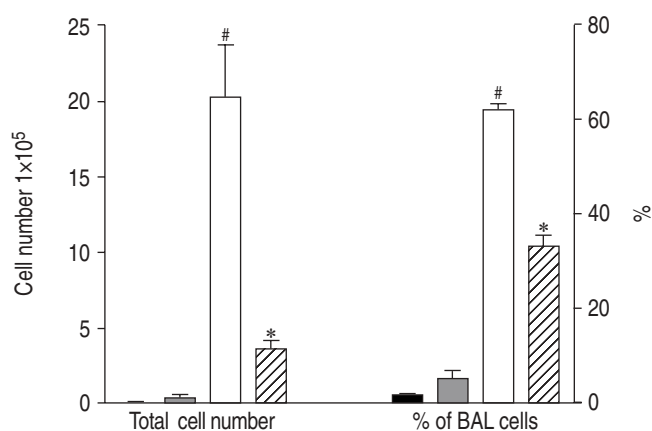asia. *Am J Respir Crit Care Med* 2003; 167: 57–64.