

Review Article

REACTIVE OXYGEN SPECIES AND AIRWAY INFLAMMATION

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Abstract—Reactive oxygen species may be generated by several inflammatory cells which participate in airway inflammation and their production may be increased in asthma. Oxygen metabolites may contribute to the epithelial damage which is characteristic of asthmatic airways and may activate cells such as mast cells in the airway mucosa. Reactive oxygen species may cause bronchoconstriction, mucus secretion, have effects on airway vasculature, and may increase airway responsiveness. The role of reactive oxygen species in airway disease has been largely neglected, but appears to be an important area for future study. It is also possible that antioxidant defenses may be defective in asthma. If reactive oxygen species participate in the inflammatory response in airway disease, then radical scavengers or antioxidants could play a useful role in therapy.

Keywords—Asthma, Airway epithelium, Bronchoconstriction, Mast cell, Eosinophil, Macrophage, Free radicals

INTRODUCTION

Oxygen-derived free radicals (superoxide anions $O_2^{\cdot-}$, hydroxyl radicals $\cdot OH$) or metabolites (hydrogen peroxide— H_2O_2 and hydrochlorous acid— $HOCl$), collectively termed reactive oxygen species, are important mediators of cell and tissue injury during inflammation and may be produced by several types of inflammatory cell. The contribution of these mediators to airway disease has been surprisingly little studied. With the promising development of effective antioxidants it may be timely to evaluate the possible role of reactive oxygen species in airway disease. Oxygen radicals have long been implicated in adult respiratory distress syndrome (ARDS) and in emphysema, where oxygen metabolites derived from inflammatory cells, such as neutrophils, may play an important role in damaging alveolar cells,^{1,2} but their role in airway inflammation is less certain.

ASTHMA AS AN INFLAMMATORY DISEASE

Asthma is characterized by variable and reversible airflow obstruction and by bronchial hyperresponsiveness, an excessive airway narrowing in response to a variety of apparently unrelated stimuli. Our understanding of the pathogenesis of asthma has changed recently and it is now viewed as a chronic inflammatory process.^{3,4} Previously, contraction of airway smooth muscle was emphasized as an important mechanism of asthmatic airway obstruction, but it is now clear that

edema of the airway wall as a result of microvascular leakage, and luminal obstruction due to exuded plasma and airway secretions, may also be contributory. Although inflammatory changes in the airway will have long been recognized as a prominent feature of fatal asthma attacks,⁵ there is now evidence from bronchial biopsies that similar, albeit less intense, inflammatory changes are found, even in the mildest of asthmatic patients.^{6,7} Several inflammatory cells are found in asthmatic airways, including mast cells, macrophages, eosinophils, neutrophils, lymphocytes, and platelets. These cells release a variety of mediators which interact in a complex manner to produce the pathophysiological features of asthma.⁸ Oxygen metabolites are almost certain to participate in this inflammatory response, although their precise role in the inflammatory response is not yet certain, and has been surprisingly neglected.

SOURCE OF REACTIVE OXYGEN SPECIES

Several of the inflammatory cells which are believed to participate in the inflammatory response of asthmatic airways have been shown to release reactive oxygen species after activation by a variety of stimuli. Activation of mast cells, macrophages,^{9,10} eosinophils,¹¹ and neutrophils¹² generates $O_2^{\cdot-}$, which are rapidly converted to H_2O_2 by superoxide dismutase (SOD); $\cdot OH$ is formed nonenzymatically in the presence of Fe_2^+ as a secondary reaction. In neutrophils myeloperoxidase also results in

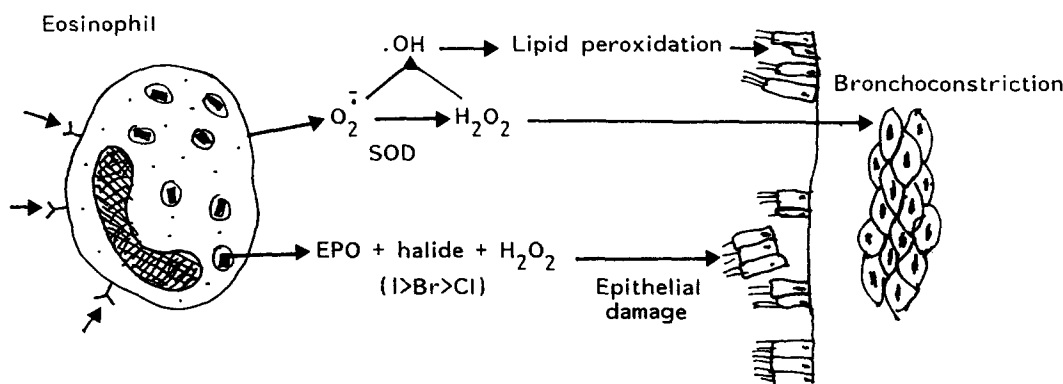


Fig. 1. Generation of reactive oxygen species from eosinophils. Activated eosinophils release superoxide anions ($O_2^{\cdot -}$) which are converted by SOD (SOD) to hydrogen peroxide (H_2O_2) and to the hydroxyl anion ($\cdot OH$) which may lead to cell damage by lipid peroxidation. Eosinophil peroxidase (EPO) interacts with H_2O_2 in the presence of halide anions to further damage epithelium. H_2O_2 also has direct bronchoconstrictor effects.

the formation of $HOCl$ from H_2O_2 in the presence of chloride ions; $HOCl$ is a potent oxidant. All of these metabolites may have effects on the various target cells of the airway.

Eosinophils play a critical role in asthma and are closely linked with bronchial hyperresponsiveness.¹³ It is likely that eosinophil products, such as major basic protein and eosinophil cationic protein, may lead to epithelial damage.¹⁴ However, oxygen metabolites generated by eosinophils may also contribute to epithelial damage (see below). Several stimuli may release $O_2^{\cdot -}$ from eosinophils, including opsonized zymosan, complement fragments, phorbol esters (via activation of protein kinase C), IgG, and IgE.¹⁵⁻¹⁷ In addition, the lipid mediator, platelet-activating factor (PAF), stimulates eosinophils to release $O_2^{\cdot -}$ via a magnesium ion dependent process.^{18,19} Eosinophils from asthmatic patients and patients with rhinitis produce more oxygen metabolites (measured by luminol chemiluminescence) in response to PAF and the stimuli than those from patients with other atopic diseases, suggesting that the eosinophils may have been "primed" to show this exaggerated response.^{20,21} The enzyme eosinophil peroxidase (EPO), which is contained in the specific granules of eosinophils²² and is released by stimuli such as PAF,²³ may play an important role in the damaging effect of free radicals. In the presence of halide ions, EPO and hydrogen peroxide form a potent cytotoxic system against a variety of cells (Fig. 1).²⁴ Thus, eosinophil peroxidase, H_2O_2 , and iodide, have a potent injurious effect on respiratory epithelial cells (type 2 pneumocytes) in vitro,²⁵ and on human nasal epithelium in vitro.²⁶

Alveolar macrophages may play an important role in introducing the late response and bronchial hyperresponsiveness which follows allergen exposure in asthmatic patients.³ These cells also generate oxygen metabolites after activation by several stimuli, including IgE through low affinity receptors.²⁷ There is evidence that alveolar

macrophages lavaged from asthmatic airways spontaneously release oxygen metabolites, measured by luminol-induced chemiluminescence, indicating that they have been activated in asthma.^{28,29} Alveolar macrophages from smokers also generate increased $O_2^{\cdot -}$, which may contribute to the airway effects of cigarette smokers.³⁰ Similarly, neutrophils from asthmatic children have been found to produce increased oxygen reactive species.³¹ A preliminary study also suggests that airway epithelial cells themselves release reactive oxygen species,³² and this might be an important source of oxygen radicals in airway disease.

Oxygen metabolites may also stimulate inflammatory cells themselves. Thus, oxygen radicals stimulate the release of histamine from mast cells.³³ Thus, the eosinophil peroxidase- H_2O_2 -halide system potentially stimulates histamine release from mast cells in a process which is both exocytotic and cytotoxic.³⁴ This suggests that oxygen metabolites which are generated by inflammatory cells may stimulate nearby cells to release oxygen metabolites in a self-perpetuating cascade.

INHALED OXIDANTS

Reactive oxygen species may also be delivered to the airways by inhalation. Exogenous oxidants may be important in exacerbation of airway inflammation. Thus, inhaled ozone may lead to an increase in airway responsiveness in animals and in normal individuals,³⁵⁻³⁷ and even ambient ozone exposure may reduce pulmonary function.³⁸ The mechanism for this increased responsiveness is not certain but, in animals, it has been linked to neutrophil infiltration in the airway epithelium.³⁹ However, the neutrophil infiltration appears to occur after the increase in responsiveness³⁷ and may be secondary to epithelial damage caused by the oxidizing effect of ozone. The increase in airway responsiveness which follows ozone exposure in guinea pigs is prevented by pretreatment with an antioxidant ascorbic

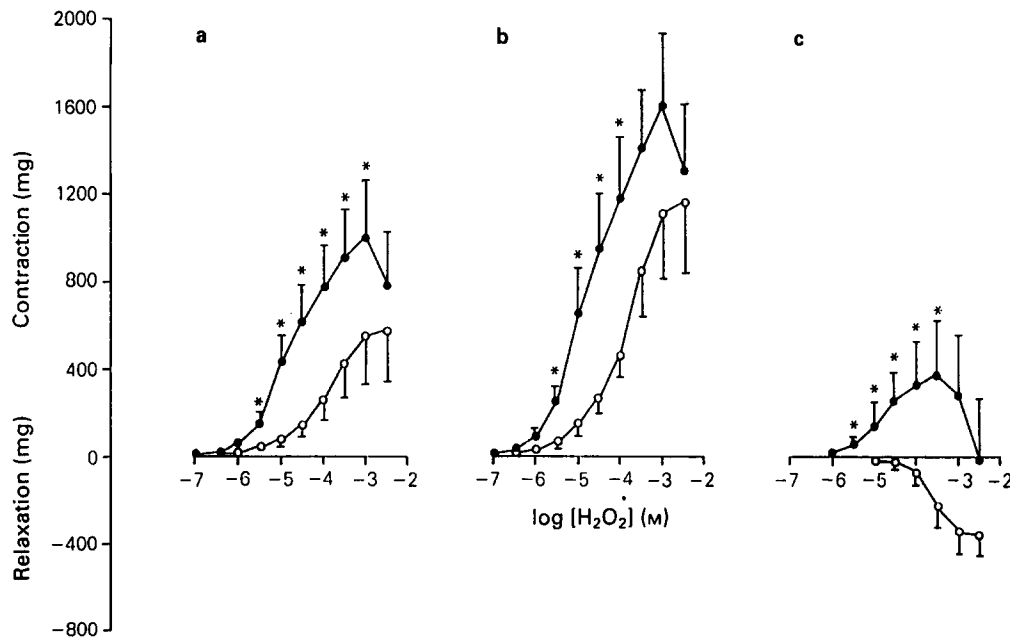


Fig. 2. Effect of hydrogen peroxide (H_2O_2) on guinea pig trachea in the presence ($\circ$) and absence ($\bullet$) of epithelium. (a) Mean values of 17 preparations are shown; (b) shows the 10/17 preparations, where contraction was observed and (c) shows 5/17 preparations where a relaxation response was observed in the intact preparation. From Rhoden and Barnes.⁵²

acid.⁴⁰ Ozone may inhibit the enzyme neutral endopeptidase in airway epithelium that normally degrades peptides such as tachykinins and bradykinin,⁴¹ thus resulting in exaggerated bronchoconstrictor responses to these peptides.

Cigarette smoking may also deliver increased oxidants to the lungs. Cigarette smoke contains many oxidizing free radicals, both in the gas phase and in tar.⁴² Cigarette smoking causes increased airway responsiveness in normal and asthmatic subjects.⁴³ Even passive smoking has been associated with an increase in airway responsiveness in children of smokers.^{44,45}

Nitrogen dioxide (NO_2) is another inhaled oxidant which may have effects on lung function. Thus, low concentrations of NO_2 increase bronchial responsiveness in asthmatic patients.⁴⁶ Inhaled oxidants associated with air pollution may therefore exacerbate existing airway inflammation in asthmatic airways.

EFFECT OF REACTIVE OXYGEN SPECIES ON AIRWAY FUNCTION

Mechanism of action

Reactive oxygen species may influence airway cells in a number of ways. Oxygen radicals are highly reactive and, when generated close to cell membranes, may oxidize membrane phospholipids (lipid peroxidation) which may continue in a chain reaction. Thus, a single $\cdot\text{OH}$ can result in the formation of many molecules of lipid hydroperoxides in the cell membrane which may

severely disrupt its function and may lead to cell death, or to damage of DNA. This may occur in epithelial cells if reactive oxygen species are generated in the airway lumen. Lipid peroxidation may also alter protein structure, thus altering antigenicity which may provoke immune responses, providing the potential for long-term changes in cell function.

Reactive oxygen species appear to oxidize certain amino acids in proteins such as methionine and cysteine, and may thus profoundly alter the function of proteins. Surface receptor proteins (e.g., beta-adrenoceptors) may be affected, leading to changes in tissue responsiveness. Enzymes (e.g., alpha-1 protease inhibitor and neutral endopeptidase) may also be inhibited, leading to profound changes in cell function. Thus, cigarette smoking has been shown to inhibit the function of neutral endopeptidase and this may result in increased airway responsiveness to substance P, which is normally degraded by this enzyme.⁴⁷ This effect of cigarette smoke is prevented by prior treatment with SOD, indicating that O_2 is involved.

Reactive oxygen species, via lipid peroxidation, may also provoke the release of arachidonic acid from membrane phospholipids and may thus lead to the release of prostaglandins and leukotrienes.^{48,49} Many of the effects of oxygen species in airways may be mediated by the secondary release of inflammatory lipid mediators.

Airway smooth muscle

There have been surprisingly few studies of the effects of reactive oxygen species on airway smooth

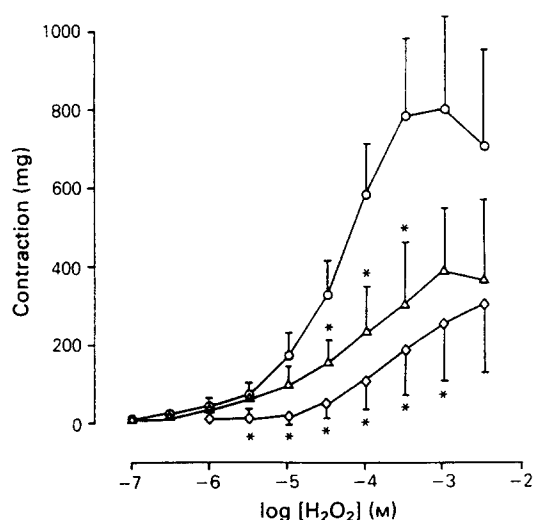


Fig. 3. The effect of catalase ($\diamond$) and mannitol ($\triangle$) on the contractile response to H_2O_2 in guinea pig trachea. From Rhoden and Barnes.⁵²

muscle function. H_2O_2 contracts canine lung parenchymal strips and bovine tracheal smooth muscle *in vitro*⁵⁰ and $\cdot OH$ contracts guinea pig tracheal smooth muscle.⁵¹ H_2O_2 (0.1 μM –3 mM) induces variable contraction in guinea pig isolated trachea since only 60% of preparations contract and most preparations relax at higher concentrations⁴⁸ (Fig. 2). The contractile response is blocked by catalase, as expected, but is also diminished

by mannitol, suggesting that $\cdot OH$ also contributes to the contractile response, as previously suggested (Fig. 3). Incubation with xanthine/xanthine oxidase, which generates $O_2^{\cdot -}$, also gives variable responses which were unaffected by SOD, suggesting that $O_2^{\cdot -}$ does not contribute to the contractile response (Rhoden, K.J.; Barnes, P.J., unpublished results).

Mechanical removal of airway epithelium potentiates contractile responses to a variety of agonists.^{53–56} It has been suggested that airway epithelial cells may release a relaxant factor in response to these spasmogens, which would therefore normally reduce their bronchoconstrictor effect.⁵⁷ Similarly, contractile responses to H_2O_2 in guinea pig trachea are potentiated by epithelial removal (Fig. 2). This could be explained by the loss of an epithelial relaxant factor or, alternatively, H_2O_2 may be inactivated by airway epithelial cells, thus reducing its effective concentrations at airway smooth muscle cells. Whether catalase is localized to airway epithelial cells is not yet certain.

Contractile responses to low concentrations of H_2O_2 are attenuated by indomethacin, suggesting that a constrictor cyclooxygenase product, such as thromboxane or $PGF_{2\alpha}$, may be released^{50,52} (Fig. 4). In an isolated perfused rabbit lung preparation oxygen metabolites have been shown to release thromboxane, which activates vasoconstriction.⁵⁸ High concentrations of H_2O_2 induce contraction in intact, but not in deepithelialized preparations, even in the presence of indomethacin,

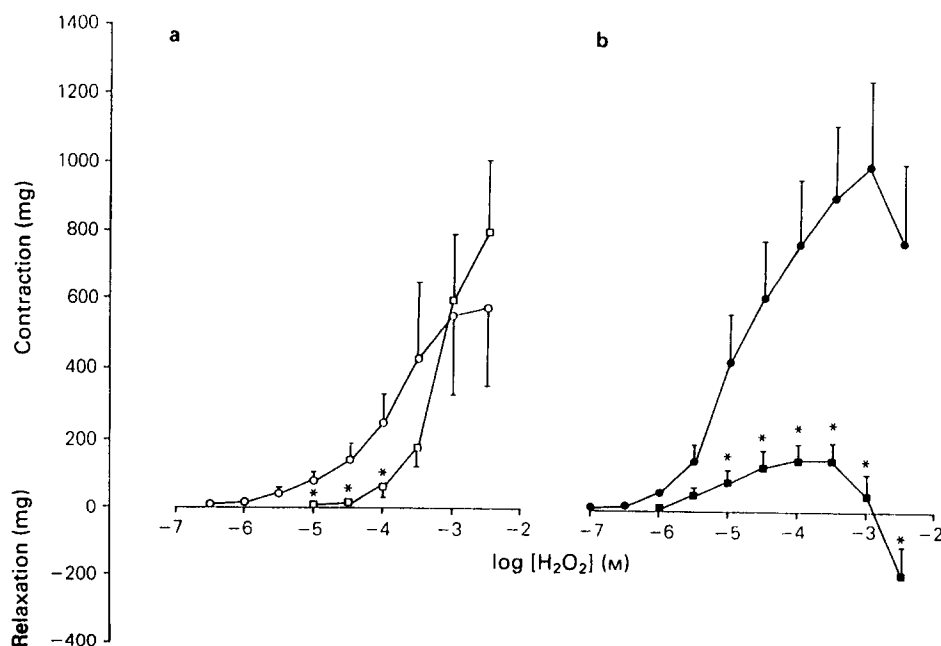


Fig. 4. Contractile responses to H_2O_2 in (a) Intact preparations and (b) preparations without epithelium in the presence (squares) and absence (circles) of 3 μM indomethacin.

EFFECT OF OXYGEN RADICALS ON AIRWAY EPITHELIAL EFFECTS

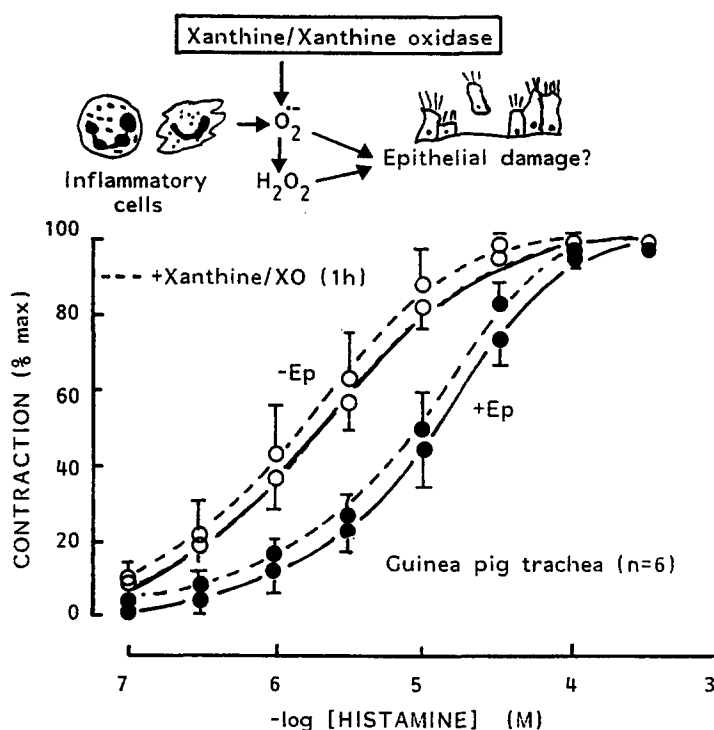


Fig. 5. The effect of xanthine/xanthine oxidase (XO) exposure (dotted lines) on the contractile response of guinea pig trachea to histamine before (●) and after (○) mechanical removal of epithelium. There was a significant potentiation of the bronchoconstrictor effect of histamine with epithelium removal, but xanthine/XO did not potentiate the bronchoconstrictor effect.

suggesting that some non cyclooxygenase product is also released from airway epithelial cells by H_2O_2 , although the nature of this constrictor is not certain. In the presence of indomethacin and epithelial removal high concentrations of H_2O_2 induce relaxation, indicating either the release from some other cell types of a relaxant or a direct relaxant effect on airway smooth muscle cells.

Effect on beta-adrenoceptors

Reactive oxygen species may impair beta-adrenoceptor function.⁵⁹ Alveolar macrophages have been shown to impair beta-adrenergic responsiveness in guinea pig trachea in vitro.⁶⁰ This has been presumed to be due to release of reactive oxygen species, since both catalase and thiouran (an $\cdot OH$ scavenger) prevent this effect. Furthermore, incubation of guinea pig lung membranes with H_2O_2 results in a reduction in beta-adrenoceptor number,⁶¹ measured by direct receptor binding, indicating a direct toxic effect, presumably via lipid peroxidation, on pulmonary beta-receptors. Direct incubation of guinea pig trachea with H_2O_2 has no effect on the response of guinea pig trachea to isoproterenol.⁵² This may be because the concentration of H_2O_2 is lower than

achieved by macrophages in close contact with airway smooth muscle, or that other inflammatory mediators released by macrophages in addition to oxygen metabolites, may be required to impair beta-receptor function. However, incubation of rat trachea with H_2O_2 causes a loss of beta-receptor sensitivity to isoproterenol which is exacerbated by depletion of selenium (to reduce glutathione peroxidase activity) and vitamin E, an antioxidant, in the diet.⁶²

In vivo inhalation of xanthine/xanthine oxidase causes bronchoconstriction in anesthetized cats, and also increased bronchial responsiveness to inhaled acetylcholine, which may reflect epithelial damage.⁶³

Airway epithelium

Epithelial shedding is a characteristic feature of asthmatic airways,⁵⁻⁷ and it is therefore possible that reactive oxygen species generated during the inflammatory response might contribute to this shedding. Purified eosinophils directly damage airway epithelial cells in vitro, after activation with platelet-activating factor, and part of this effect appears to be due to oxygen metabolites, since it can be markedly reduced by catalase.⁶⁴ In vitro,

however, generation of oxygen radicals with xanthine/xanthine oxidase has no effect on the responsiveness of guinea pig airway smooth muscle to histamine, implying that airway epithelium has not been damaged significantly (Rhoden, K.; Barnes, P.J., unpublished) (Fig. 5). In vitro it may not be possible to achieve the high local concentrations of oxygen metabolites which would be present in the vicinity of an epithelial cell, however. Recent studies have demonstrated that, when guinea pig tracheal epithelium is exposed to H_2O_2 , there is increased transit of labeled terbutaline across the epithelium,⁶⁵ suggesting that there may be separation of epithelial cells, allowing more rapid transit of certain molecules. This appears to correlate with histological changes to airway epithelial cells.

Airway secretion

Reactive oxygen species stimulate the release of high molecular weight glycoconjugates from cultured guinea pig airway epithelial cells and tracheal explants in vitro.⁶⁶ This increased mucus secretion appears to be dependent on cyclooxygenase products, since it can be inhibited by indomethacin. Such a mechanism might explain the hypersecretion of mucus which occurs after inhalation of the oxidant ozone in sheep trachea.⁶⁷ Ozone also increases the release of eicosanoids from bovine tracheal epithelial cells.⁶⁸ Perhaps exogenous oxidants in the form of cigarette smoke or endogenous reactive oxygen species from airway inflammatory cells might contribute to the mucus hypersecretion which occurs in chronic bronchitis and asthma, respectively.

Effects on vessels

Oxygen metabolites may have potent vascular effects. H_2O_2 potentially relaxes pulmonary vascular smooth muscle in vitro,⁶⁹ but in vivo causes pulmonary vasoconstriction through the release of thromboxane.⁵⁸ Oxygen radicals also cause increased vascular permeability, possibly via a direct damaging effect on vascular endothelial cells.⁷⁰ Similar studies in the bronchial circulation have not been reported, but it seems likely that oxygen radicals may lead to microvascular leak and edema in airways, if they damage endothelial cells in the same manner. Whether they lead to vasodilation or vasoconstriction cannot be predicted, however.

Effects on inflammatory cells

Oxygen radicals may themselves have effects on inflammatory cells. They may stimulate formation of chemotactic factors from arachidonic acid⁷¹ and may activate mast cells to release histamine.³² This might explain how ozone inhalation leads to neutrophil infil-

tration into airways.³⁵ The effects of reactive oxygen species on mediator generation and on other inflammatory cells is worthy of further investigation, in view of the potential for a feed-forward mechanism whereby cells which produce oxygen metabolites may then stimulate neighboring cells to produce more.

ROLE IN ASTHMA?

It is difficult to be certain of the role of reactive oxygen species in asthma. Many of the inflammatory cells which are believed to be activated in asthmatic airways are known to release oxygen radicals. Enhanced oxygen radical release has been demonstrated in alveolar macrophages,^{27,28} circulating neutrophils,³⁰ and eosinophils²⁰ of asthmatic patients. Indeed, there is a correlation between H_2O_2 production from stimulated neutrophils and the degree of bronchial hyperresponsiveness in asthmatic children³⁰ and in patients with chronic airflow limitation.⁷²

Defect in antioxidant defenses?

There are several antioxidant defense mechanisms which protect against reactive oxidant species, and include the enzymes SOD, catalase, and glutathione peroxidase (which removes H_2O_2 by oxidation of reduced glutathione to oxidized glutathione). Whether these enzymes are defective in asthmatic airways has not been studied, but if they are localized to airway epithelial cells any epithelial shedding which may occur in asthmatic airways may enhance oxidant injury by reducing antioxidant defenses. Glutathione peroxidase blood concentrations are indeed reported to be lower in asthmatics with food and aspirin intolerance.⁷³ Glutathione peroxidase is the only human enzyme which requires the trace element selenium as a cofactor and has a selenocysteine residue at its active site.⁷⁴ It is of interest, therefore, that in asthmatic patients there is a reduction in whole blood selenium concentrations and glutathione peroxidase concentrations compared to nonasthmatic controls.^{75,76} New Zealand has a high prevalence, mortality, and morbidity from asthma,⁷⁷ and this may be related to deficient dietary intake of selenium, reflecting the low selenium content of the soil.⁷⁶

Evidence for reactive oxygen species release

Oxygen metabolites have only a transient existence and therefore cannot be measured directly in vivo. In a recent study an attempt to measure oxygen metabolites in asthma was made by measuring serum concentrations of phospholipid-esterified 9,11-linoleic acid and the parent 9,12-linoleic acid.⁷⁸ 9,11-linoleic acid is thought to arise from the naturally occurring parent compound 9,12-

linoleic acid by free radical attack and appears to be a specific and relatively stable marker of the generation of reactive oxygen species.⁷⁹ thus, elevated plasma concentrations of 9,11-linoleic acid have been reported in rheumatoid arthritis and in alcoholic patients. No evidence for a similar increase in levels in blood was found in acute severe asthma, however, but this by no means excludes local airway production.

Another way of studying the contribution of oxygen radicals is to investigate the effects of free radical scavengers. Currently available scavengers are not very efficient, but ascorbic acid, which is an antioxidant, reduces methacholine-induced bronchoconstriction in asthmatic subjects,⁸⁰ although this could be mediated by other mechanisms. More potent free radical scavengers may be necessary to investigate the role of reactive oxygen species in asthma.

CONCLUSIONS

Although reactive oxygen species are generated by several of the inflammatory cells implicated in asthma, and increased production of oxygen radicals has demonstrated from various inflammatory cells isolated from asthmatic patients, their role in asthma has been surprisingly neglected. Oxygen metabolites may have potent damaging effects on cells such as airway epithelium, thus promoting increased airway responsiveness to inflammatory mediators, and may also release mediators from inflammatory and epithelial cells, but may, in addition, contract airway smooth muscle directly. If certain oxygen species contribute to the inflammatory response in asthma, then oxygen radical scavengers or antioxidants could play a useful role in therapy. The development of effective antioxidants in the future may therefore be of value in the treatment of airway inflammation.

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