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COMMENTARY

The Antioxidant Milieu at Asthmatic Respiratory Tract Surfaces

Commentary on the article by Schock *et al.* on page 375

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Childhood atopic asthma is a respiratory tract disease of unusually high and increasing prevalence, representing a leading disease entity resulting in emergency room or hospital admission. It is characterized by reversible airway obstruction, airway hyperreactivity and airway infiltration by inflammatory-immune cells, particularly eosinophils and mast cells, but also lymphocytes and neutrophils. Recent therapeutic strategies have focused upon the importance of anti-inflammatory treatments in the amelioration of the clinical manifestations of this condition (1, 2). Sites of inflammation are rich in reactive oxygen species (ROS) contributed by phagocyte NADPH oxidases and other oxidant-producing enzymatic systems. Abundant evidence documents that oxidative (and nitrosative) stress exists at nearly all sites undergoing inflammatory reactions (3), suggesting that oxidative pathways amplify tissue injury at these sites. It is not surprising, then, that antioxidant therapy has been proposed for many inflammatory lung diseases, including asthma (4, 5).

ROS, whether generated by exogenous or endogenous (including inflammatory-immune) sources, are potentially toxic to cells by virtue of their ability to disrupt redox homostasis and to "damage" critical biomolecular species (3, 6). As such, organisms have evolved antioxidant defense mechanisms to cope with excessive pro-oxidant challenges. Respiratory tract lining fluids (RTLFs) and their underlying respiratory tract epithelial cells (RTECs) form the first line of lung defense against potentially toxic inhaled environmental oxidants and endogenous inflammatory-immune oxidant products. Together, these occupy a central place in airway pathophysiology. RTLFs are highly complex and heterogeneous fluids which contain high-molecular weight, thiol-rich, antioxidant mucopolypeptide glycoproteins (mucus), low molecular weight antioxidants such as uric acid and glutathione, antioxidant micronutrients (*e.g.* ascorbate and vitamin E), iron binding proteins (*e.g.* lactoferrin and transferrin) and small quantities of antioxidant enzymes (4, 7–9). Together they provide for a complex "controlled" redox balance at RTEC apical surfaces.

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Importantly, the spectra of RTLF antioxidants vary considerably among upper airway nasal passages, tracheobronchial airways and alveolar surfaces (7). There is a paucity of information regarding the transport systems and turnover of RTLF antioxidants, their relationships to RTEC small MW antioxidants and antioxidant enzyme systems, and the role that newly described transport systems (*e.g.* ascorbate (10),) may play in maintaining either RTLF or RTEC small MW antioxidant pools.

The increasing awareness that airway inflammation represents a key factor in asthma has led to the implementation of successful guidelines for anti-inflammatory-directed therapies (1, 2). However, since inflammation is accompanied by oxidative stress, what about antioxidant therapies? The complex roles that antioxidant therapies might play at inflammatory sites are worthy of discussion. It is a commonly (but somewhat controversially) accepted paradigm that allergen-induced airway inflammation is, at least in part, orchestrated by activated Th2 lymphocytes (11). Macrophages, eosinophils, neutrophils, dendritic cells, other lymphocytes, mast cells, fibroblasts, epithelial cells and smooth muscle cells all claim their positions in airway inflammatory processes as well. The concept that antioxidants might influence asthmatic pathobiology is evidenced by the fact that Th1 and Th2 cytokines are differentially regulated under conditions of oxidative stress (12), that apoptotic death pathways, known to be activated in asthmatic RTECs and important in the clearance of airway phagocytes (13), are known to be modulated by antioxidants (6), and that antioxidants may decrease phagocyte and nonphagocyte NADPH oxidase activities (14) and affect the production of nitric oxide (NO) (15).

Further complexities include the fact that NO levels themselves may modulate Th1/Th2 and apoptotic responses (16), and that phagocyte or nonphagocyte ROS production may elicit a broad array of physiologic responses, from cell proliferation to gene expression and apoptosis, not only within phagocytes, but also in neighboring cells (17). Therapeutic antioxidants, especially those which affect intracellular thiol/disulphide relationships (18, 19), may modulate numerous discrete redox-sensitive signaling pathways requiring transient

oxidation for their function, thus having fundamental effects on almost all aspects of lung cell biology (6, 20). Given the myriad of potential effects, it is difficult to predict a theoretical construct for the efficacy of therapeutic antioxidants in asthma.

Many factors need to be taken into consideration in constructing a rational construct for antioxidant therapies in asthma. These would include: (i) the increasing recognition of the role of pleiotropic nonphagocytic NADPH oxidases in generating localized intracellular signaling levels of ROS which influence the complicated network of signaling cascades responsible for gene regulation and protein metabolism (6, 20); (ii) the increasing appreciation for the role of the antioxidant micronutrients, including vitamin C (21, 22) and vitamin E (23), in mediating functions seemingly unrelated to their actions on redox potential, including pathways related to immunomodulation and gene expression; (iii) the fact that NO pathways, known to be dysregulated in asthma (24), have important modifying roles of ROS at many redox active sites modulating a wide variety of cellular functions (3, 25, 26); and (iv) the fact that certain "antioxidants." including polyphenolics and chemoprevention agents, may actually function as mild pro-oxidants which influence cellular protective and adaptive antioxidant systems (*e.g.* enhance GSH-related pathways, phase II detoxifying/metabolizing pathways and/or other antioxidant enzyme systems). The observation that at least one redox-regulated extracellular antioxidant enzyme system may be adaptively increased in asthmatic lungs (27) supports the importance of increased local antioxidant defenses in asthmatic airways.

Schock and colleagues, in this issue of Pediatrics Research, address several issues of oxidant stress in childhood asthma (28). In 78 asymptomatic atopic asthmatic children undergoing intubations for elective surgery and using the sampling technique of nonbronchoscopic bronchoalveolar lavage (BAL), they obtained samples of distal RTLFs to assess selected antioxidants and markers of oxidative stress. A history of intermittent wheeze and an elevation of serum IgE levels were used to define atopic asthma. BAL cell counts and differentials, ascorbate, uric acid and vitamin E levels, and markers of protein and lipid oxidation were used to assess the distal RTLF inflammatory and antioxidant milieu, and their results were compared with those obtained in 124 nonasthmatic children reported earlier (29).

As expected, the asthmatic children had a higher proportion of BAL eosinophils (3x) and mast cells (4x). Interestingly, BAL ascorbate, urate and α-tocopherol concentrations were similar to those in nonasthmatic children, as were the markers for BAL protein oxidation (carbonyls) and lipid peroxidation (TBARS). The authors made a number of statistical correlations in the asthma group, the most impressive of which was the correlation between the number of inflammatory cells in BAL (especially the number of eosinophils in BAL) and the extent of the BAL protein carbonyl content. These associations did not reach significance in samples from nonasthmatics. A weak but significant association between the protein carbonyl and TBARS content of BAL was noted, suggesting phagocyte oxidations of both protein and lipid constituents of this fluid. When comparing BAL and plasma micronutrient antioxidant concentrations between the asthmatics and nonasthmatics, no important differences were apparent. Curiously, no associations between individual plasma levels of ascorbate and their BAL concentrations were present, possibly because of the wide variability in the dilution of the distal RTLFs by the lavage maneuvers and/or the relatively good nutritional status of the population studied in which the range of intake of antioxidants and variances of plasma ascorbate were rather small. The degree to which RTLF (and RTEC) ascorbate concentrations at all RT levels (*e.g.* airways especially) can be influenced by ascorbate intake and/or plasma ascorbate concentrations remains an important unanswered question.

This provocative paper involves formidable technical and logistical obstacles, yet focuses attention on evidence for antioxidant micronutrient deficiencies and/or oxidative stress in the BAL fluids of atopic asthmatics in clinical remission. A significant "take-home" message is that, in asymptomatic children with minimal evidence of airway inflammation in the BAL compartment, there were essentially no measured indices that collectively suggested either deficiencies in antioxidant micronutrients or evidence of oxidative stress. However, there is tantalizing subgroup evidence that excessive inflammatory cells, most notably eosinophils, were associated with protein oxidation and possibly lipid peroxidation. This finding is reminiscent of other studies showing an association between neutrophil accumulation and higher amounts of protein and lipid oxidation products in other airway diseases including premature, very low birth weight infants, a population at high risk for developing airway disease (30). Of interest, many of these infants appear to have subnormal BAL ascorbate concentrations (31), suggesting that steps to minimize oxidative stress may be efficacious in this disorder (32, 33).

The studies of Schock *et al.* provoke a host of other issues pertinent to nutritional antioxidants and asthma. Although it can be assumed that asthmatic children with no history of a recent exacerbation will have near-normal baseline FEV_1 s and PEFRs (34), it would have been useful to have characterized the relationships between the BAL antioxidant/oxidant profiles and the physiologic correlates of asthma (*e.g.* airway obstruction and/or reactivity) and BAL lymphocyte subgroups and cytokine profiles (including the pro-oxidant cytokines). In the absence of physiologic and relevant immunologic correlates of active disease, the reported findings are not unexpected, although it should be noted that atopic asthmatics in apparent clinical remission have shown increased inflammatory cells in bronchial biopsies, emphasizing that inflammatory-oxidative events reflective in BAL may not be concordant with events occurring in airway tissues (35). The Schock *et al.* studies emphasize that in a relatively homogeneous youthful population, the redox milieu of the RTLFs, as measured by ascorbate, urate and vitamin E levels, appears not to bear a clear relationship with the incidence of atopic asthma. An intriguing finding in the present study was that 59% of the atopic asthmatic children had exposure to environmental tobacco smoke, as did 57% of the controls, suggesting a higher proportion of Irish children are "trapped" within the smoking environment than in the United States. In this small data set the odds ratio for smoke exposure and asthma and wheeze was not increased, although

odds ratios pooled from meta-analysis in several large epidemiologic studies are usually in the order of only 1.2 to 1.3 (36). Recently, the severity of asthma in children has been linked to their exposure to environmental tobacco smoke (37).

The recent literature presents a convincing argument that acute asthma is accompanied by oxidative and nitrosative stress in the RTLFs (5, 38–40). At least one study has reported a rigorous marker of lipid peroxidation (isoprostanes) to be elevated in the plasma of asthmatics, and to be related to the severity of the asthma, but unaccompanied by any differences in plasma antioxidant vitamins (41). Nevertheless, several epidemiologic studies have linked dietary antioxidants and asthma incidence/severity (42–45) and some intervention studies in subjects with a high exposure to oxidative air pollutants have shown short-term protective effects of antioxidants on lung function (46–49). Of note, a recent study suggests that antioxidant supplementation (50 mg/d of vitamin E and 250 mg/d of vitamin C) might modulate the impact of ozone exposure on the small airways of children with moderate to severe asthma living in Mexico City (50). However, thus far, few studies have addressed the implementation of antioxidant strategies to the treatment of acute asthma beyond the concept that anti-inflammatory treatments are paramount. Since inflammation begets oxidative stress, treatments designed to prevent and/or treat inflammation are also addressing, to a degree, the issue of oxidative stress.

In conclusion, the concept of antioxidant therapy for asthma and other respiratory diseases (*e.g.* COPD) is of substantial current interest (51), buttressed by observational studies suggesting a role of a healthy "antioxidant" diet along existing guidelines for the prevention of coronary heart disease and cancer (42–45). It is clear that additional research is needed to build mechanistic constructs to more adequately depict how these therapies might work in the inflammation-related lung diseases including asthma. Such studies will have to include those designed to delineate the complex interplay between the small molecular weight antioxidants, intracellular constitutive and adaptive antioxidant enzyme systems, and host inflammatory-immune and reparative processes including airway remodeling. This research will need to be integrated alongside clinical observational studies such as on page ___ of this issue.

Further experimental studies will need to test the concept that antioxidants can be: (i) effectively delivered to respiratory tract surfaces; (ii) prevent harmful oxidations of extracellular and/or intracellular biomolecular species; and most importantly (iii) useful in the prevention and/or treatment of the clinical manifestations of asthma. Understanding of the integrative role of antioxidant species in lung health will continue to provide increased insights into extracellular and intracellular redox states as major players in lung disease pathobiology. This knowledge will have extrapolated value not only to the asthma community, but also to a host of other lung diseases characterized by ongoing inflammation accompanied by increases in ROS released by phagocytes, including bronchopulmonary dysplasia, cystic fibrosis, infant and adult respiratory distress syndromes, interstitial pulmonary fibrosis and COPD (4, 51).

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