Oxidants, Antioxidants, and Respiratory Tract Lining Fluids

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Respiratory tract lining fluids (RTLFs) are a heterogeneous group of substances covering the respiratory tract epithelial cells (RTECs) from nasal mucosa to alveoli. Antioxidants contained in the RTLFs can be expected to provide an initial defense against inhaled environmental toxins. The major antioxidants in RTLF include mucin, uric acid, protein (largely albumin), ascorbic acid, and reduced glutathione (GSH). RTLF antioxidants can be augmented by such processes as transudation/exudation of plasma constituents; RTEC secretory processes, including glandular mucus secretion; and cellular antioxidants derived from lysis of RTECs and of inflammatory cells. The antioxidant composition of RTLFs and their role in modulating normal and pathophysiologic RTEC functions under conditions of oxidative stress are yet to be fully characterized. — Environ Health Perspect 102(Suppl 10):185–191 (1994)

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Introduction

The respiratory tract lining fluids (RTLFs) form an interface between the underlying respiratory tract epithelial cells (RTECs) and the external environment. The RTLFs thus constitute a "first line of defense" against inhaled toxic gases, such as SO_2, O_3, NO_2, and tobacco smoke. Constituents of the RTLFs may detoxify pollutants to protect the underlying RTECs. For example, it has been suggested that inhaled O_3 and NO_2 react with RTLF components and may never reach the underlying cells (*1–3*). Another possibility is that toxic actions in RTECs are mediated by products of reaction of inhaled toxins with RTLFs, e.g., lipid hydroperoxides or aldehydes.

Interest in the role of RTLF antioxidants has been stimulated by observations that uric acid is secreted by the same upper RTECs that secrete mucin (*4,5*), that α-tocopherol is secreted by lower RTECs together with surfactant (*6*), that bronchoalveolar RTLFs contain ascorbic acid and reduced glutathione (GSH) in considerable excess of plasma levels (*7–9*), and that mucin itself shows antioxidant properties *in vitro* (*10–12*). Interest has been further aroused by the observation that the GSH content of the lower RTLF appears to be subnormal in AIDS (*13*), in idiopathic pulmonary fibrosis (*14*), in cystic fibrosis (*15*), and in adult respiratory distress syndrome (ARDS) (*16*). Indeed, perhaps variations in the composition of RTLFs might help to explain interspecies variability in sensitivity to respiratory tract injury by inhaled toxins (*17–19*).

Although there are abundant data concerning the reactivity of air pollutants (e.g., O_3) with molecules in isolation (*20–28*), there are many fewer data concerning targets of damage in complex biologic systems such as the RTLFs (*29,30*).

Oxidant Effects on Plasma

As a first step in examining the spectrum of reactions that can be expected to occur when complex biologic fluids (such as RTLFs) are exposed to O_3, we have used plasma as a target (*31,32*). Plasma is easier to obtain than RTLFs and contains a wide range of antioxidants (both aqueous and in the lipid phase) (*33–36*). One special advantage is that the antioxidants present are well defined (*33*). Of course, it is not an ideal model, in that it does not consist of separate sol and gel layers, and mucin and surfactant are missing.

Injury to the lung, e.g., by high concentrations of toxic gases, can be expected to cause neurogenic inflammation, with transudation of plasma constituents into the RTLFs (*37,38*), i.e., they become closer to plasma in composition (Figure 1). Indeed, it has been proposed that this transudation, often used as a marker of injury and inflammation, also is beneficial in that it will increase total antioxidant capacity of the RTLFs (*40,41*). Hence, exposure of plasma to O_3, followed by measurements of consumption of antioxidants in relation to oxidative protein modification and the appearance of lipid hydroperoxides (*32*), has provided important clues in understanding O_3 reactions in complex biomolecular systems. These studies have significant implications in that *in vitro* exposures of tissue culture systems to O_3 should consider the contributions of reactions of O_3 with constituents of the culture media.

Problems with RTLFs

Why are RTLFs' antioxidant compositions so badly characterized when compared with plasma? First, the technique of bronchoalveolar (and nasal or airway) lavage produces considerable and variable dilution of the RTLFs (*42–45*).* Second, the composition of RTLFs varies in different levels of the respiratory tract. For example, as shown in Table 1, RTLF depth (both gel and sol layers) in the upper respiratory tract may range from 1 to 10 μm

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*For purposes of this article and in keeping with convention, RTLF refers to the lining fluids of the entire respiratory tract, whereas epithelial lining fluids (ELF) refers to that fluid present on the bronchoalveolar (the peripheral RTLF) surfaces that is obtained by bronchoalveolar lavage.

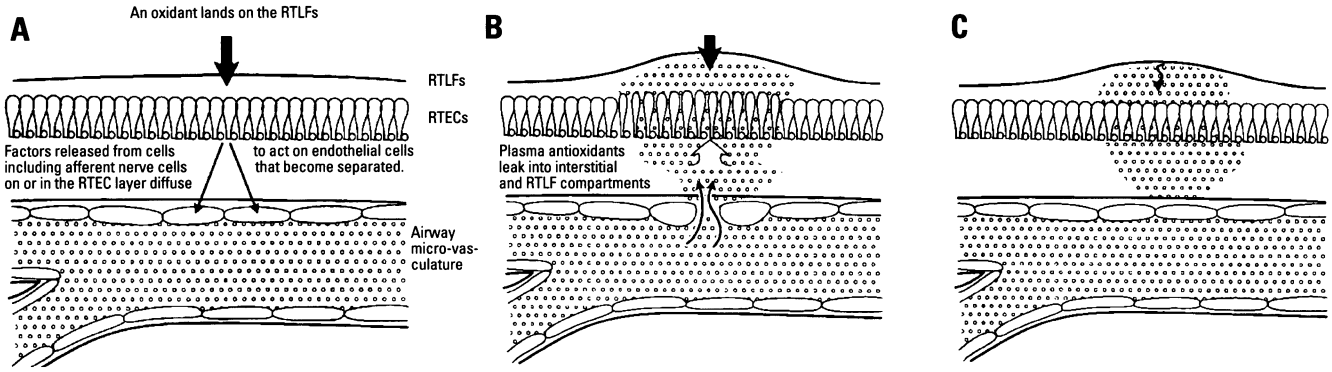

Figure 1. Role of airway plasma exudation in providing antioxidant substances to the RTLFs. (*A*) Initiation by direct "activation" or injury to RTECs or by activation of neurohumoral mediator pathways; (*B*) endothelial permeability increase allows plasma constituents to move into the interstitial space with subsequent "leak" into RTLFs, possibly via hydrostatic pressure-operated mechanisms that transiently open valvelike paracellular RTEC pathways (*39*); (*C*) plasma antioxidants augment RTLF antioxidants.

Table 1. Human RTLF.[a]

	Thickness, μm	Surface area, cm^2	Volume, ml	Turnover
Nasal	5–10	180	0.15	?>1000 ×/day
Airways	1–10	4500	3.5	?>20 ×/day
Alveoli	0.5 – 0.2	885,000	9	???

RTLF, respiratory tract lining fluid. [a]Based largely on morphologic data (*19*).

and mucin is present, whereas in the distal bronchoalveolar regions RTLF depth is only 0.2 to 0.5 μm and surfactant is uniquely present (*19,46,47*). Third, the quantitative description of antioxidants present at any given level of the respiratory tract is compromised by the complex gel-sol nature of the upper RTLFs and the possibility that the mucin-containing gel layer is covered by a lipid layer that may be derived in part from the lower bronchoalveolar regions (*48*).

Considerations of RTLF constituent turnover, mucus secretion rates, RTEC and inflammatory–immune cell desquamation and death, airway microvasculature transudative/exudative processes and interactions with inflammatory–immune cells and adjacent RTECs all contribute to the complexity of evaluating the antioxidant efficiencies of RTLFs. Even RTEC cilia, which constitute a sizeable portion of RTLF volume and RTEC surface area (*49*) and whose loss does not necessarily impair the viability of the ciliated cell, could make some contribution to overall RTLF antioxidant capabilities, both by providing proteins and lipids that could act as sacrificial targets of damage by toxic agents and by release of ciliary cytoplasmic constituents into the RTLFs, thereby protecting more important functional targets.

The fact that cigarette smokers produce abnormally large amounts of mucus (*50*) and appear to have unusually high amounts of uric acid, glutathione, and ascorbic acid in their bronchoalveolar fluids (*51,52*) may, for example, contribute to their reported decreased sensitivity (assessed spirometrically) to O_3 (*53*).

What Do We Know?

As in other extracellular fluids (*33–35*), the antioxidants in RTLFs can be expected to be variable in nature (Table 2), consisting of low molecular mass antioxidants, metal-binding proteins, certain antioxidant enzymes, and sacrificial reactive proteins and unsaturated lipids. The oxidant dose reaching the target RTEC, is presumably influenced by the volume (including surface area and thickness), composition and turnover rate of the various antioxidants present in the RTLFs (*18,54*).

Table 3 presents approximate calculations of the likely antioxidant levels in the peripheral RTLFs (ELF). Plasma values (known with much greater certainty) are shown for comparison. Bearing in mind the great uncertainty of these ELF calculations due to the variability and uncertainty of the dilution factor, it is nevertheless clear that levels of certain antioxidants contained in ELF are higher than in plasma. Most notable is the considerably higher concentration of GSH in ELF, as compared with plasma, and the lower concentrations of protein-SH. Ascorbic acid levels appear higher in ELF, but only a few measurement in humans have been made, and even fewer simultaneous plasma and ELF have been reported.

Table 4 shows that the antioxidant composition of RTLFs varies between different animal species (and probably between different strains of the same species, e.g., rats) and this must be borne in mind when extrapo-

Table 2. Antioxidant species in RTLFs.

Low molecular weight antioxidants
Metal-binding proteins
Antioxidant enzymes
Sacrificial reactive proteins and unsaturated lipid in RTLFs

RTLF, respiratory tract lining fluids.

Table 3. Approximate values for the concentrations of nonenzymatic antioxidants in human plasma as compared to peripheral RTLF (epithelial lining fluid).

Antioxidant	Plasma,[a] μM	ELF,[b] μM
Ascorbic acid	40	100
Glutathione	1.5	100
Uric acid	300	90
Bilirubin	10	–
α-Tocopherol	25	2.5
β-Carotene	0.4	–
Ubiquinol-10	0.6	–
Albumin-SH	500	70

RTLF, respiratory tract lining fluids; ELF, epithelial lining fluid. [a]Plasma values derived from Sies et al. (*33*), Halliwell et al. (*34*), and Frei et al. (*35*). [b]ELF values largely derived from Hatch (*19*). When derived from bronchoalveolar lavage (BAL) fluid data, it has been assumed that ELF is 100 × more concentrated than BAL fluid.

Table 4. Comparison of estimated levels of certain antioxidants in epitelial lining fluid from different mammals.[a]

	Guinea pig, μM	Rat, μM	Human, μM
Ascorbic acid	600	2000	160
Glutathione	220	130	165
Uric acid	53	46	129
α-Tocopherol	0.8	0.6	2.5
Protein-SH	150	75	60

[a]Data are calculated from the results of Slade et al. (*17,18*) and Hatch et al. (*19*) by assuming that both human and rodent lavages are 100 × diluted, that lavage proteins are 50% albumin, and that albumin-SH represents the sole protein-SH present in bronchoalveolar lavage fluid.

lating the results of animal exposure to oxidizing air pollutants to human exposures.

Table 5 illustrates the variability in antioxidant composition between human nasal lining fluid and peripheral RTLFs (ELF). Note the higher levels of uric acid in the nasal fluids. Since uric acid reacts quickly with O_3, it has been suggested that uric acid acts as a "scrubber" of inhaled O_3 in the nose, perhaps protecting the deeper parts of the respiratory tract by diminishing the amount of inhaled O_3 that is delivered there (*4,5,32,56*).

Table 6 lists the protein antioxidants that have been reported to be present in the peripheral RTLFs (ELF). Although some investigators have considered catalase (*57–59*) to be physiologically important in the ELF, there remains uncertainty whether these enzyme systems play a significant role in the antioxidant defenses of RTLFs. Due to its high concentration in upper RTLFs, it is likely that lactoferrin plays a significant antioxidant function by binding iron and inhibiting iron-dependent free radical reactions (*5,34*).

Table 5. Approximate concentrations of selected antioxidants in nasal lining fluid compared to bronchoalveolar lining fluids (ELF).[a]

	Nasal lining fluid, μM	ELF, μM
Ascorbic acid	40	100
Uric acid	160	90
Glutathione	40	100
Albumin-SH	10	70

ELF, epitelial lining fluid. [a]Nasal fluids derived from Peden et al. (*5*), Hatch (*19*), and Greiff et al. (*55*). ELF derived from Slade et al. (*17,18*) and Hatch (*19*). In data derived from nasal lavages or bronchoalveolar lavages, 40× and 100× dilution factors have been assumed, respectively (*5,19*). Total protein assumed to be 20% albumin in nasal lavage and 50% albumin in ELF (*19*).

Table 6. Antioxidant proteins known to be present in RTLFs.[a]

Metal-binding proteins	Other antioxidant proteins
Lactoferrin	Catalase
Ceruloplasmin	SOD
Transferrin	Glutathione reductase
Albumin	Glutathione peroxidase
	Ceruloplasmin (ferroxidase activity)
	Albumin (e.g., –SH activity)

RTLF, respiratory tract lining fluids. [a]These proteins either bind metal ions in safe forms unable to catalyze damaging free radical reactions or possess other antioxidant properties (*34,57,59*).

Comments about Specific Antioxidant Substances in RTLFs

Uric Acid

Uric acid has been identified recently as a major low molecular weight antioxidant present in upper RTLFs, in amounts that approximate those of plasma (*5,60*). Airway gland cells appear to take up plasma or interstitial uric acid and secrete it along with lactoferrin into the RTLFs (*5*). Owing to its cosecretion with mucin, uric acid seems predestined to alleviating free radical/oxidant attack on not only other constituents of RTLFs but also on RTECs. Indeed, uric acid appears to be the most potent scavenger of O_3 in plasma (*32,61*) and undoubtedly plays an important role in protecting the respiratory tract from the oxidant effects of O_3 (*32,56*). However, without knowledge of such important factors as uric acid turnover and the possible reactivity of its oxidation products, e.g., allantoin (*62*), it is difficult to quantitate the precise antioxidant contribution of uric acid to the upper RTLFs.

Mucin

Mucins consist of a core protein, rich in serine and threonine, to which carbohydrates are attached, and cysteine-rich domains, many of which are involved in disulfide formation, that along with the carbohydrates, contribute to its viscous properties (*48,63*). Mucins have metal binding properties (*64*), effectively scavenge •OH (*10*) and would be expected to scavenge HOCl/OCl^- (*65,66*) because of their abundance of –SH and S–S moieties (*63*). After inhalation of a toxicant, they are secreted in increased amounts and may represent a major antioxidant in the upper RTLFs, both because of their own intrinsic antioxidant properties and because substances intermixed with mucin (e.g., lipid, protein, DNA) that are also contained in the mucus (gel layer), could represent sacrificial antioxidants. There is a need to determine the quantitative extent to which RTLF mucin and the nonmucin constituents of the mucous gel contribute to the detoxification of inhaled toxins. This is an especially important consideration in that it is the mucous layer that forms the initial interface for reaction with inhaled toxins (*2,3*).

Ascorbic Acid

Ascorbic acid is an important water-soluble extracellular fluid antioxidant (*67*), important not only because it scavenges neutrophil oxidants but also because it may reduce oxidized vitamin E (*68*), thus restoring the antioxidant capability of this important lipid antioxidant. It appears to be actively secreted in its reduced form into the lower RTLFs (*59*), at least in experimental animals (17,18,69). It is an unsettled issue as to whether this is true in humans, because too few data exist.

Several observations support an antioxidant role for ascorbic acid in RTLFs. The decreased systemic ascorbic acid levels and its increased turnover seen in cigarette smokers appear to be mainly caused by increased RTLF ascorbic acid utilization subsequent to its oxidation by oxidants contained in the gas phase of cigarette smoke (*59,70,71*). Further support comes from the observation that ascorbic acid appears to protect against O_3^- (*72*) and NO_2^- (*73*) induced airway hyperresponsiveness as well as against nonspecific (*74*) and virus-associated (*75*) increases in airway responsiveness. This is perhaps not surprising in that O_3 and NO_2 both rapidly oxidize extracellular fluid antioxidants (*32,76*) and that all four conditions are associated with airway inflammation, that in itself induces an oxidative stress and is associated with airway hyperreactivity (*77*). It is probable that arachidonic acid metabolites and/or nitric oxide could be involved (*77,78*).

Vitamin E

The lipid-soluble antioxidant vitamin E plays a key role in protecting lipid contained in aqueous dispersions (micelles, lipoprotein particles) and in biologic membranes from peroxidative injury, largely by its ability to reduce peroxyl radicals (*79,80*). Many investigators have shown that suboptimal amounts of vitamin E produce a heightened lung susceptibility to oxidant injury (*24*). Since the main function of vitamin E is to protect biomembranes where it is intercalated into membrane structures, it is not clear how the contained vitamin E protects the lipid constituents of the RTLFs including surfactant (*59*). However, it is known to be present in the RTLFs, and its concentration is thought to be decreased in lower RTLFs obtained from cigarette smokers (*51,80*), presumably because of the decreased lipid surfactant known to be present in smokers and possibly because of increased utilization. The latter argument is buttressed by the finding that one of its metabolites, vitamin E quinone, is increased in lower RTLFs of smokers (*80*).

It is probable that the interaction between ascorbic acid and the free radical from vitamin E (*68*) constitutes a major interactive antioxidant system in the lung at the interface between RTLFs and RTECs.

Thiols

Thiols, particularly GSH, are capable of scavenging •OH, H_2O_2, HOCl, and other oxidative products of phagocytes (*65*). The fact that decreased levels of bronchoalveolar lavage GSH have been found in several diverse lung diseases (*13–16*) has encouraged therapeutic strategies to increase RTLF levels of GSH, especially by aerosolization of GSH into the respiratory tract (*81,82*). Another thiol, *N*-acetylcysteine, also has found use as an approach to augment RTLF antioxidant capabilities and in some studies appears to have produced small rises in RTLF GSH (*83*). However, several conditions associated with inflammatory airway responses, including cigarette smoking (*51,52*), asthma (*84*), and experimental exposure to O_3 (*85*), are associated with high levels of RTLF GSH. Perhaps this represents an adaptive response to the oxidant stress of cigarette smoking or the inflammatory response (*52*), or perhaps it occurs subsequent to GSH release from inflammatory cells or RTECs that have been injured or shedded. Indeed, it has been demonstrated that toxic particles, such as quartz and asbestos, cause GSH releases from alveolar macrophages (*86*) and RTEC shedding is known to occur in most forms of lung inflammation including asthma (*87*).

It should be remembered that thiols are also potentially toxic, often by interacting with transition metal ions or by reacting with oxygen radicals to form reactive thiyl or oxysulfur radicals (*88–90*).

The availability of transition metal ions in normal RTLFs may be very limited because of the presence of antioxidant metal ion-binding proteins (*57,59,91*) (Table 6). However, oxidative stress itself can often cause metal ion release. For example, it has been reported that constituents of cigarette smoke can mobilize iron from ferritin and that ferritin levels are increased in smokers (*92*), and catalytic iron is present in the bronchoalveolar fluids of cigarette smokers (*93*). Further studies are needed to evaluate the therapeutic usefulness of inhaled or systemically administered thiols under conditions of respiratory tract oxidative stress, including clinical conditions characterized by inflammatory–immune reactions.

Indeed, an ancillary therapeutic intervention could be to inhibit the reactivity of such transition metals (especially iron and copper) by using suitable chelating agents. This may, for example, be appropriate in cystic fibrosis patients, most of whom have airway infections with *Pseudomonas aeruginosa*. To enhance its rate of obtaining and transporting iron, *P. aeruginosa* secretes the siderophores pyochelin and pyoverdin. Thus, in addition to the endogenous iron chelators lactoferrin and transferrin, RTLFs of cystic fibrosis contain bacterial siderophores. In the presence of neutrophil oxidants and proteases derived from both Pseudomonas and neutrophils, these iron-containing proteins could be expected to release bound iron, that in the presence of neutrophil oxidants could result in iron-catalyzed formation of •OH (*94–97*).

Piecing Together a "Big Picture"

The information above underscores the lack of complete understanding of the extent and significance of oxidant/antioxidant interactions in RTLFs. Research is critically needed to define the extent of RTLF reaction with environmental air pollutants and the physiologic effects of these changes on the inflammatory–immune and epithelial cells that are in intimate contact with this fluid. Experiments such as direct determinations of products generated from biologic targets and from antioxidants during their action [e.g., those produced when uric acid scavenges reactive oxygen species (*62,98*)], direct measurements of lipid peroxidation products (*29*), and detailed measurements of oxidized protein and glycoprotein are important. A complicating question will be to determine the precise role of cell contents released as a result of cell desquamation, cytolysis, and death. These products will include heme proteins and transition metal ions (prooxidants), antioxidants such as GSH, and enzymes such as SOD and catalase. The mucus layer is another complicating factor.

Another gap in the big picture concerns the interaction between antioxidants in the RTLFs and those contained in the RTECs. Why are RTLF GSH levels much higher than plasma and why are ascorbic acid levels significantly higher? Do epithelial cells secrete GSH? Perhaps they absorb GSSG, reduce it, and release GSH. Perhaps the same is true for ascorbic acid. Oxidized ascorbic acid could be taken up by the cells, reduced to ascorbate, and released back into the RTLFs. What are the relationships among the levels of antioxidant agents in RTLFs, the intracellular antioxidants, and the susceptibilities to oxidative stress of the adjoining RTECs? At the present time, there exists only limited information on this important subject.

The Way Forward

The case for an important role of reactive oxygen species in mediating some of the effects of environmental pollutant gases is substantial. Despite intriguing data concerning cellular oxidative stress and adaptive responses to both the primary oxidative insult and the secondary oxidative insults due to tissue injury and inflammatory-immune processes (which are themselves difficult to distinguish), there exists a need to broaden the way in which we think about respiratory tract oxidative damage to encompass more specific mechanistic end point determinations. Examples of unresolved problems include the mechanism and the pathophysiologic importance of the decreased GSH levels noted in BAL fluids obtained from patients with cystic fibrosis (*15*), idiopathic pulmonary fibrosis (*14*), ARDS (*16*), and HIV (*13*).

An obvious need exists for more experiments to investigate the role of RTLF antioxidants in respiratory tract injury. The challenge is to conduct studies that provide critical insights into the relationships between oxidative stress in RTLFs and parameters of RTEC function. Among the potential important research areas that need addressing are the following:

- Improved methods for measuring all of the following in both upper and lower RTLFs: levels of antioxidants and products of free radical attacks upon them; rate of production of reactive oxygen species at the plasma membrane surface of the RTECs; biomolecular markers of oxidative damage to antioxidants, proteins, lipids, and DNA and the effects of such products (e.g., protein radicals, aldehydes, and lipid hydroperoxides) on RTEC function.
- The role of redox active metal ions, whether bound or free, in RTLF oxidations, and the possible release of such metal ions under conditions of oxidative stress secondary to environmental toxin exposure or to inflammatory–immune processes.
- The temporal and causal relations between environmental pollutants, the primary oxidative stress they can cause, and the secondary oxidative stress due to tissue injury and inflammatory–immune processes.
- Development of both animal and clinical models to test the clinical effects of altering RTLF antioxidant defense systems, thereby influencing RTEC susceptibility to inhaled environmental pollutants.
- Determinations of the precise interac-

tions between antioxidants in RTLFs and those of RTEC plasma membranes. For example, could extracellular GSH help maintain α-tocopherol levels in RTEC plasma membranes?

- The relationship between oxidative stress in RTLFs and RTECs and coexisting or subsequent development of RTEC dysfunction.
- The possible attenuation of damage by environmental air pollutants using antioxidants, e.g., administered in aerosol form (*81,82*), systemically (*83*), or in the diet (*99*).

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