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Oxidative stress: acute and progressive lung injury

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Oxidative stress in lung often occurs in humans during acute lung injury (ALI) and in the acute respiratory distress syndrome. The lung inflammatory response may proceed to the development of pulmonary fibrosis, a devastating complication that occurs in premature infants after prolonged exposure to high oxygen concentrations. Oxidant-related ALI can be induced by airway deposition of lipopolysaccharide or IgG immune complexes, resulting in activation of recruited neutrophils and residential macrophages, whose oxidants and proteases produce reversible ALI. In the presence of a powerful trigger of leukocytes (phorbol myristate acetate), or following intrapulmonary deposition of enzymes that generate oxidants, extensive endothelial and epithelial damage and destruction occurs, overwhelming repair mechanisms of lung and resulting in pulmonary fibrosis. How residential or circulating stem cells participate in regeneration of damaged/destroyed cells may provide clues regarding therapy in humans who are experiencing lung inflammatory damage.

Keywords: acute lung injury; pulmonary fibrosis; oxidants; antioxidants; inflammation; macrophages

Introduction

It is well known that oxidant production within lung can lead to acute lung injury (ALI) and sometimes progressive lung injury which often results in irreversible fibrosis.[1–4] The sources of oxidants may be extrinsic or intrinsic. Agents such as paraquat, when inhaled, can be metabolized into free radicals which may be intensely lung-damaging often resulting in pulmonary fibrosis.[5] Intrinsically generated oxidants may be derived from mitochondria, but the most important source of damaging oxidants is phagocytic cells (residential macrophages and recruited neutrophils [PMNs]) that can generate toxic oxygen metabolites from assembly on cell surfaces of NADPH oxidase (also known as NOX2). Similar pathways leading to oxidant generation can occur in endothelial and alveolar epithelial cells, but the majority of oxidants in lung arises from stimulated phagocytic cells.[6,7]

Defenses against toxic oxygen metabolites in lung include superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione, which is present in mM concentrations in lung lining fluids.

These antioxidant enzymes are inducible in a variety of situations such as during hyperoxia, in the presence of lipopolysaccharide (LPS), etc.[8,9] Enzymes such as SOD can be induced in lung by hyperoxia, bacterial LPS, to name just a few examples. SOD dismutes the superoxide anion ($O_2^{\bullet}$) to H_2O_2. Catalase converts H_2O_2 to H_2O, while glutathione peroxidase is a selenium-containing enzyme that catalyzes conversion of H_2O_2 to H_2O in the presence of glutathione, which is then converted to its oxidized form, GSSG. The presence of these antioxidant enzymes and reducing factors in lung illustrates how the lung adapts to maintain a redox balance and can respond to oxidizing conditions that may threaten the structural and functional integrity of the lung. It is understandable why the lung is so responsive to such threats, because it is the single organ in the body that is exposed to the highest concentrations of O_2. In the following, we describe how enzyme and substrate combinations as well as inflammatory events in lung featuring oxidant production by phagocytes can be used to evaluate under what conditions oxidant production in lung results in outcomes.
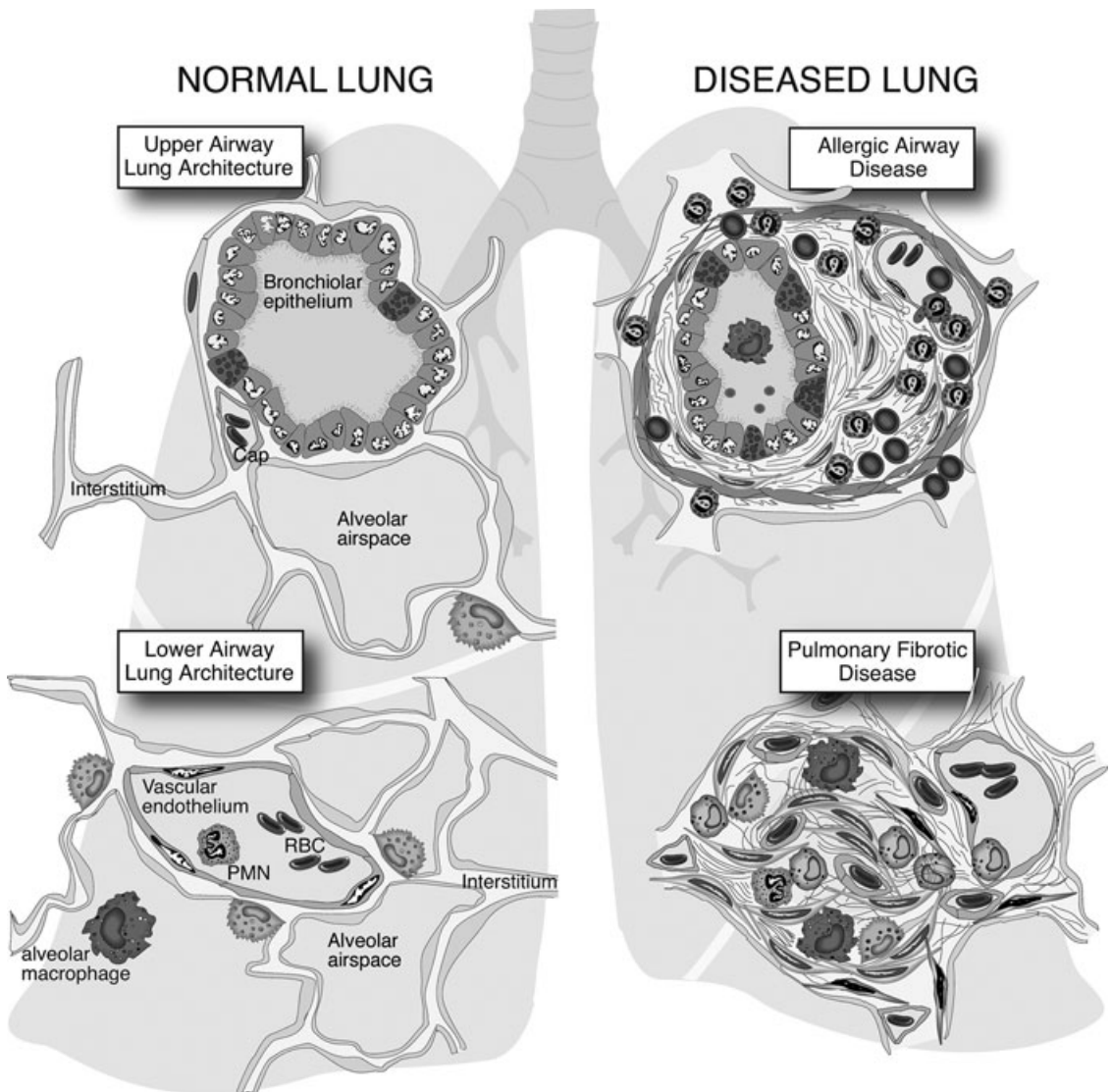
Figure 1. Representation of upper and lower airways of normal lung (left) and inflammatory changes in upper airways of asthmatic lung (upper right) and fibrosis and inflammatory cells in distal airways (lower right).

Inflammatory responses involving upper and lower airways

The architecture of normal upper and lower airways is schematically shown in Figure 1 (left upper and lower frames). Upper airways contain bronchioles lined by ciliated epithelial cells and occasional goblet cells that produce mucous, all being surrounded by smooth muscle cells. The distal (lower) airways contain capillaries, the interstitial compartment and the alveolar compartment, which is lined by alveolar

epithelial cells (Types I and II) (left lower frame). In disease states such as asthma (upper right frame), the upper airways are altered with the appearance of inflammatory cells (eosinophils, mast cells, lymphocytes, and occasional neutrophils [PMNs]) in the wall, greatly increased amounts of smooth muscle cells and large amounts of mucous secreted into the airway lumen. When oxidant injury overwhelms the repair capacity of the distal airway compartment (lower right frame), alveolar collapse may result together with extensive fibrotic scars, which effectively
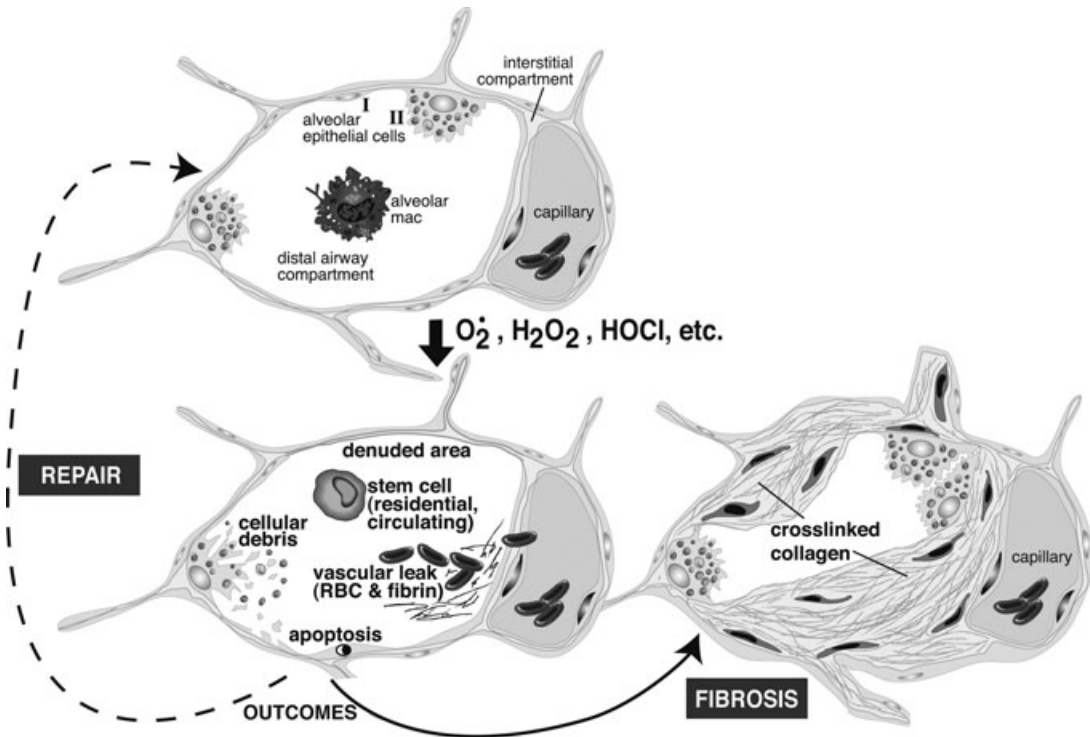
Figure 2. Schematic of events before (upper frame) and after instillation of PMA into rat lungs (lower frames). If injury can be reversed by regenerative capacities of lung (lower left frame), the lung is restored to the preinjury state. If injury is too intense, the result will be pulmonary fibrosis (lower right frame).

destroy the air exchange between affected airways and the capillary system.

Generation of intrinsically generated lung oxidants

Oxidants can be generated by lung macrophages and recruited PMNs. Although certain chemicals (e.g., paraquat) are metabolized into highly toxic products in lung, we will restrict our comments to oxidants generated by phagocytic cells in the lung or by enzyme/substrate combinations delivered to lung. Toxic oxygen products can be generated when macrophages or PMNs undergo stimulation by factors such as bacterial LPS or IgG immune complexes (IgGIC). Toxic oxygen products (superoxide, $O_2^{\bullet}$; hydrogen peroxide, H_2O_2 or its peroxidative products [e.g., hypochlorous acid, HOCl]) can be intercepted by naturally occurring or induced antioxidants such as SOD or catalase. Those antioxidant enzymes are present naturally in lung but the intrapulmonary levels of these enzymes can be greatly elevated when the lung faces a large burden of oxidants (such as hyperoxia) that compromise the re-

dox balance of lung.[8] It is possible with phorbol myristate acetate (PMA) to very powerfully activate lung macrophages, which overwhelms the antioxidant reserves of the lung and results in catastrophic parenchymal damage,[9] as described below. In the case of LPS or IgGIC induced injury, lung damage is limited and reversible, and the lung architecture is restored to normal in a matter of a few days.[10,11] In the case of PMA, damage is so intense as to overwhelm the repair and regenerative abilities of lung, leading to pulmonary fibrosis. Under such conditions, there is extensive damage/destruction of alveolar epithelial cells and vascular endothelial cells, resulting in intense alveolar edema ("alveolar flooding") and intraalveolar hemorrhage. The reversibility or irreversibility of ALI seems to be due to the extent of damage/destruction of vascular endothelial and alveolar epithelial cells. In the latter case, lung residential or blood borne stem cells may enhance the regenerative capacity of the lung (Fig. 2). If this capacity is overwhelmed, there will be collapse of alveoli and development of dense cross-linked collagen, which will, in the same locale, ablate gas

exchange between the alveolar compartment and the capillaries.

Acute and progressive lung injury after lung exposure to PMA

As indicated above, airway instillation of PMA (20 μg) results in intense parenchymal damage of the lung due to intense damage/destruction of endothelial cells and alveolar epithelial cells. The sources of these damaging oxidants are lung macrophages in the absence of PMN recruitment. Damage or destruction of endothelial and epithelial cells of the distal airways results in the breakdown of both barriers, with intense hemorrhage and flooding of the alveolar compartment, together with diffuse fibrin deposition (as described later). Such types of outcomes can be greatly attenuated in the presence of catalase but not SOD, suggesting that the toxic oxidants are H_2O_2 and/or its peroxidative products such as HOCl. Interestingly, the development of PMA-induced ALI is not affected by PMN depletion in blood, supporting the interpretation that lung macrophages are the source of the highly damaging oxidants.[9]

## Lung injury resulting from extrinsic, oxidant-generating enzymes

ALI can also be induced by introducing into the distal airway system appropriate oxidant-generating enzymes. This strategy allows one to further define the nature of the most lung-damaging oxidants. A powerful oxidant generating system includes the use of glucose and glucose oxidase (which generate H_2O_2) in the presence of peroxidases (lactoperoxidase, myeloperoxidase). The combination of xanthine and xanthine oxidase generates $O_2^{\bullet}$. The permeability index was employed as the endpoint (albumin leak into lung as a sensitive and quantitative endpoint of ALI). The combination of xanthine and xanthine oxidase resulted in a nearly 10-fold increase in injury, suggesting that $O_2^{\bullet}$ may be directly injurious to lung. When the combination of glucose and glucose oxidase was used to generate H_2O_2, albumin leak was barely double the background level. On the other hand, in the copresence of either lactoperoxidase or myeloperoxidase, the albumin leak was increased five to ninefold over background levels. The expected products from either peroxidase in the presence of H_2O_2 would be hypohalides, such as HOCl, HOBr, and HOI. These

data suggest that H_2O_2 *per se* is not especially toxic, but that its products generated by a peroxidase are the key toxic products damaging the lung. Such data are consistent with many reports indicating that nucleated mammalian cells can withstand mM concentrations of H_2O_2 without exhibiting evidence of damage.[12,13]

The toxic products generated by glucose, glucose oxidase and lactoperoxidase. In the copresence of catalase, injury dropped by approximately 95%, which is consistent with the conclusion that peroxidative products of H_2O_2 represent the key toxic products. As expected, SOD did not affect lung damage generated by glucose, glucose oxidase and lactoperoxidase, because no generation of $O_2^{\bullet}$ would be expected. Not shown in is the fact that PMN depletion had no protective effects on lung injury generated by either xanthine + xanthine oxidase or by glucose + glucose oxidase + lactoperoxidase, all of which indicates that recruitment of PMNs into lung under such conditions does not contribute significantly to the oxidant burden in lung.

Morphology of oxidant-mediated ALI

Morphological features of ALI following intrapulmonary generation of oxidants following airway instillation of glucose, glucose oxidase (28 units), lactoperoxidase (1.7 units) were evaluated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). As shown in Figure 3 employing SEM, normal lung (frame A) contained smooth intact alveolar walls in which capillaries could be identified. In some areas small projections from the alveolar walls could be seen, representing alveolar type II epithelial cells. Four hours after initiation of lung injury (frame B), the morphology was dramatically different. Alveolar spaces contained RBCs, together with tenuous white fibrous collections of fibrin. In some areas these collections were confluent, resulting in thick fibrin deposits. In other areas, discontinuities due to dropout of alveolar epithelial and vascular endothelial cells could be seen. 48 h after the onset of injury (frame C), extensive collections of fibrin remained together with leukocytes and RBCs. By 5 days (frame D) residual RBCs were still present in alveolar spaces, which were undergoing collapse, associated with residual fibrin and thick bands of collagen (frame E and
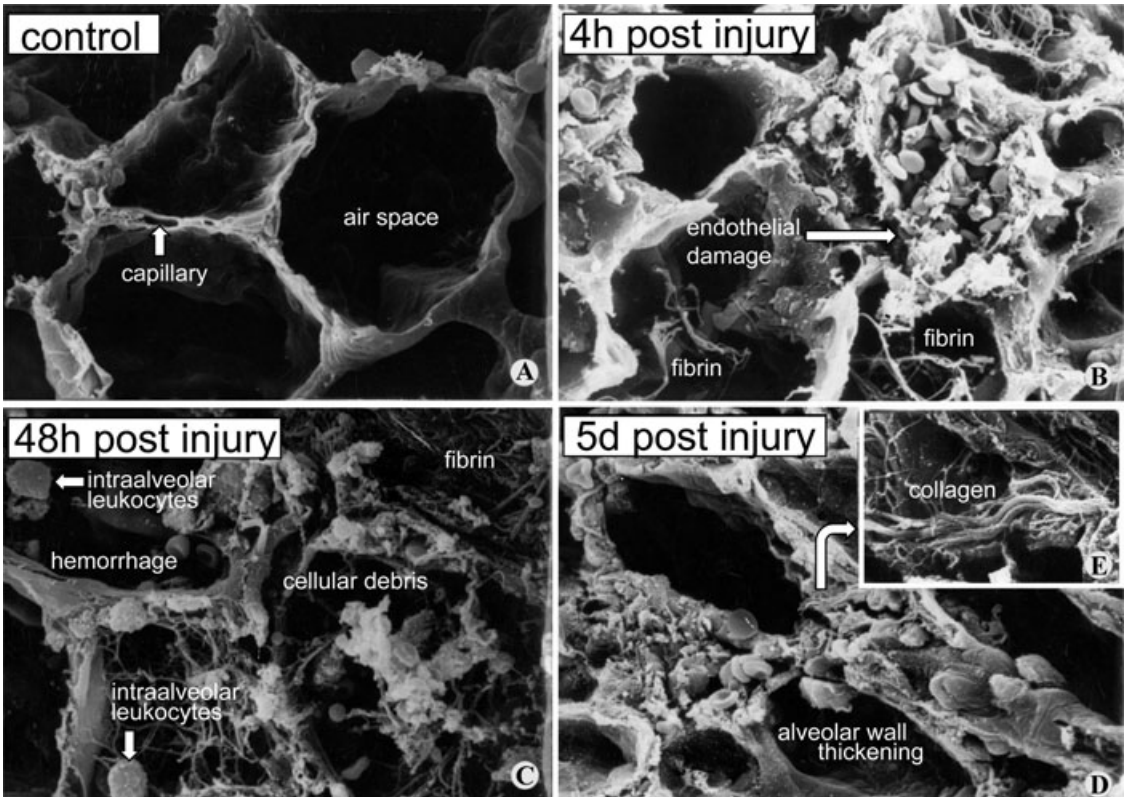
Figure 3. Scanning electron micrographs of control lung and lungs 4 and 48 h after airway instillation of glucose, glucose oxidase, and lactoperoxidase (see text for details) (1100×).

inset in frame E). Regenerating alveolar epithelial cells could also be seen.

Based on TEM analysis of tissues from the same lungs and at the same timepoints (Fig. 4), frames A, B (4 h) showed extensive cell debris, platelets in capillaries along the endothelial surfaces, and the presence of RBCs in alveolar spaces, together with extensive fibrin deposits and evidence of edema fluid, protein, and some PMNs. Also, disintegrating cells (alveolar epithelial cells) within the alveolar compartment were found, and denuded alveolar membranes were also evident. Forty-eight hours after injury (frame C), extensive intraalveolar hemorrhage, edema fluid, and fibrin deposits were evident in alveolar spaces. In addition, disintegration of alveolar walls and extensive cellular debris were evident. By day 5 (frame D), extensive collagen could be seen in the lung parenchyma together with collapse of alveolar spaces, prominence of fibroblasts and evidence of proliferating Type II alve-

olar epithelial cells. In the same areas prominent regenerating Type II alveolar epithelial cells were present.

Discussion

These data indicate that ALI resulting from generation of intrapulmonary oxidants derived from either activated lung macrophages or from oxidant generating enzymes delivered into the lung can induce ALI that may be reversible or irreversible (the latter resulting in pulmonary fibrosis). Reversibility occurs when oxidants are derived from phagocytic cells (macrophages and PMNs) and when the stimulant (e.g., LPS, IgGIC) results in limited oxidant generation. On the other hand, if the stimulus (such as PMA) is especially powerful, it appears that macrophage generated oxidants overcome the limit of natural antioxidant enzymes in lung (catalase, SOD, and glutathione peroxidase) as well as constitutive GSH. Under such conditions the regenerative
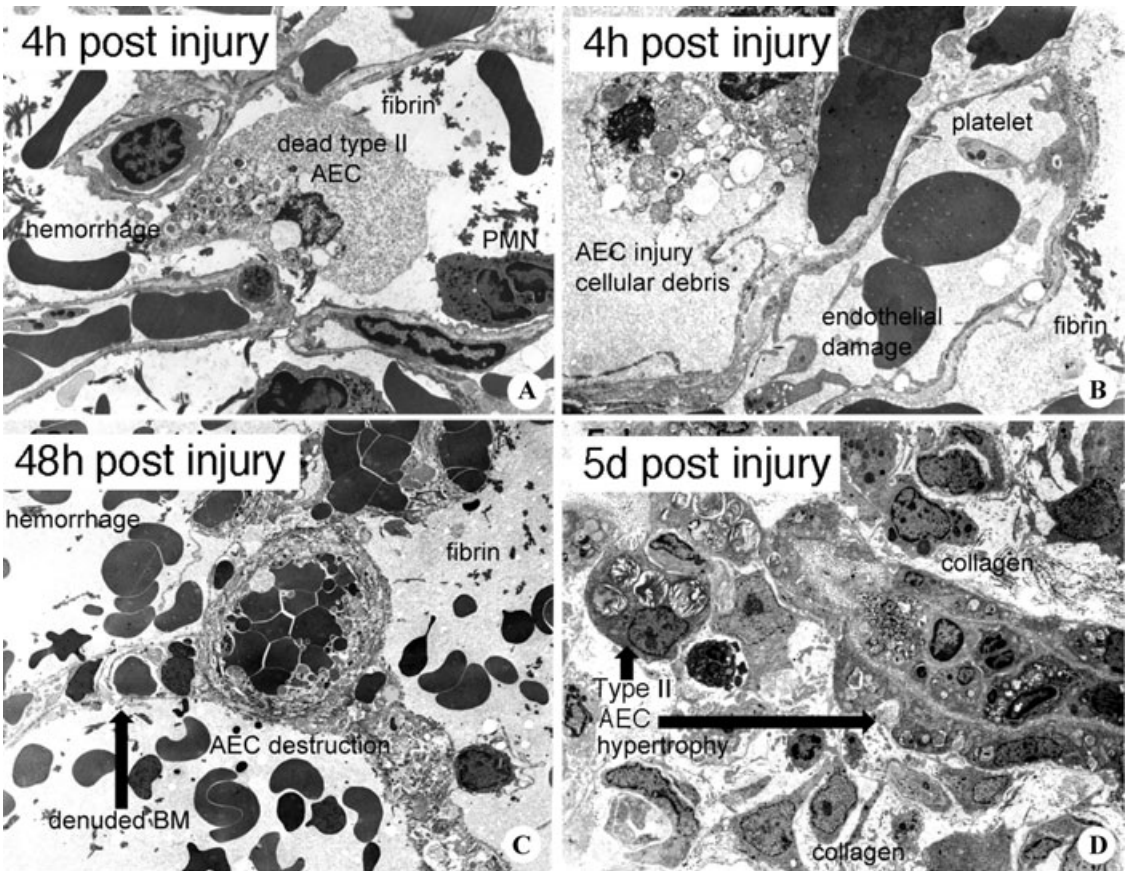
Figure 4. Transmission electron micrographs of lungs 4 and 48 h and 5 days following the same treatments described in legend to Figure 3 (Frame A, 3200×; B, 5000×; C, 1650×; D, 1300×).

capacity of lung is overwhelmed and the outcome is development of pulmonary fibrosis.

The use of extrinsic enzymes (glucose oxidase, xanthine oxidase, and lactoperoxidase) has allowed a provisional determination of the oxidants that are especially lung damaging. It appears that $O_2^\bullet$ generated by xanthine oxidase + xanthine can be intensely lung-damaging, although it is difficult to relate this to $O_2^\bullet$ produced by macrophages and PMNs in lung, because $O_2^\bullet$ is rapidly converted to H_2O_2 by SOD and can also interact with $^\bullet NO$. The production of H_2O_2 by glucose oxidase + glucose, not surprisingly resulted in very limited lung damage, because *in vitro* studies usually show that mM levels of H_2O_2 are required to produce cell damage.[12,13] However, the presence of lactoperoxidase or myeloperoxidase, which converts H_2O_2 into organochloride, organiodine, and organochloride products which are extremely lung damaging prod-

uct(s) and can lead to irreversible lung injury and pulmonary fibrosis (Figs. 3 and 4). Because MPO is naturally present in substantial quantities in PMNs and can be readily secreted, it seems likely that HOCl may be the lung-damaging product that leads to irreversible lung damage and fibrosis in the experimental protocols employed.

Conclusions

It is well known that oxidant generation in lung can be highly lung damaging. Natural sources of oxidants in lung are macrophages and recruited neutrophils. In most cases, limited oxidant production from these phagocytes allows the development of reversible lung injury. However, an extremely strong stimulus of phagocytes (PMA) may overwhelm the regenerative capacity of lung, with resultant parenchymal collapse and fibrosis. Use of substrates and enzymes allows the probing of the

most toxic oxidants in the lung setting. Reagents such as xanthine and xanthine oxidase or glucose plus glucose oxidase in the copresence of lactoperoxidase or myeloperoxidase can severely damage the lung beyond its regenerative capacity, resulting in a fibrotic outcome. Understanding the dynamic between oxidant production in lung and engagement of antioxidant defenses provide information relevant to oxidant-mediated damage in human lungs.

Conflicts of interest

The author declares no conflicts of interest.

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