State of the Art

Superoxide Dismutases in the Lung and Human Lung Diseases

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The lungs are directly exposed to higher oxygen concentrations than most other tissues. Increased oxidative stress is a significant part of the pathogenesis of obstructive lung diseases such as asthma and chronic obstructive pulmonary disease, parenchymal lung diseases (e.g., idiopathic pulmonary fibrosis and lung granulomatous diseases), and lung malignancies. Lung tissue is protected against these oxidants by a variety of antioxidant mechanisms among which the superoxide dismutases (SODs) are the only ones converting superoxide radicals to hydrogen peroxide. There are three SODs: cytosolic copper–zinc, mitochondrial manganese, and extracellular SODs. These enzymes have specific distributions and functions. Their importance in protecting lung tissue has been confirmed in transgenic and knockout animal studies. Relatively few studies have been conducted on these enzymes in the normal human lung or in human lung diseases. Most human studies suggest that there is induction of manganese SOD and, possibly, extracellular SOD during inflammatory, but not fibrotic, phases of parenchymal lung diseases and that both copper–zinc SOD and manganese SOD may be downregulated in asthmatic airways. Many previous antioxidant therapies have been disappointing, but newly characterized SOD mimetics are being shown to protect against oxidant-related lung disorders in animal models.

Keywords: free radicals; oxidants; antioxidants

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OXIDANT STRESS OF THE LUNG

Lung represents a unique tissue for oxidant stress among most organs because it is directly exposed to higher oxygen tensions. Thus, local oxygen partial pressure at the alveolar level is much higher than in other vital organs such as heart, liver, and brain. Oxygen pressure in the inhaled air is 20 kPa (150 mm Hg) and is ~13.3 kPa (100 mm Hg) at the alveolus, but it is ~6 kPa (45 mm Hg) in $\bar{v}$ blood and may be as low as 1 mm Hg in some sites within other organs. Another special feature of the lung is its large surface area (~70 m^2 in the adult human lung).

Because of their direct exposure to ambient air, lung cells experience enhanced oxidant stress by environmental irritants and pollutants including oxidants such as cigarette smoke, ozone, and free radical–generating environmental carcinogens. In addition, a typical component in most lung disorders and lung infections is inflammation and activation of inflammatory cells with consequent free radical generation. Oxygen therapy is an important but problematic issue in the treatment of prematurely born neonates and in the respiratory insufficiency associated with the acute respiratory distress syndrome. Several therapies, most importantly chemotherapy and radiation, lead to free radical–induced tissue damage where again the lung is the most commonly involved organ. Thus, lung represents a unique tissue exposed not only directly to higher oxygen tensions and environmental oxidants but also to oxidants produced by a variety of lung diseases and in the course of their therapies.

The first reactive oxygen species produced in the reduction pathway of oxygen to water is the superoxide anion, which participates in the generation of other toxic metabolites, most importantly hydrogen peroxide (H_2O_2), hydroxyl radical, and peroxynitrite (Figure 1). Most living cells, including lung cells, generate free radicals under normal conditions nonenzymatically via autooxidation. The primary sites of oxygen radical production are the electron transport chain in the mitochondria, and cytoplasmic and membrane-bound oxidant-generating enzymes (e.g., nicotinamide adenine dinucleotide phosphate reduced oxidase, nitric oxide synthase, and xanthine oxidase) (1). The generation of

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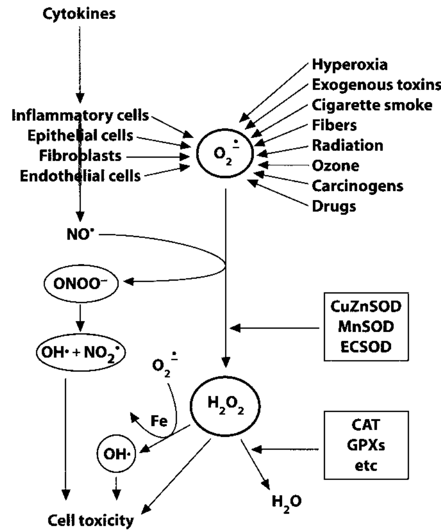

Figure 1. Source of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the lung. Generation of ROS and RNS by environmental agents and by various cells and the initial reactions of superoxide (O_2^-), hydrogen peroxide (H_2O_2), and nitric oxide (NO·) to generate reactive species that cause cell and tissue injury. Most environmental toxins generate ROS both directly and by activating inflammatory cells. This results in the activation of inducible nitric oxide synthase with generation of NO· and other RNS, and activation of myeloperoxidase (MPO) with formation of halogenated ROS and RNS metabolites (not shown). The most important antioxidant mechanisms related to these pathways include superoxide dismutases (SODs), glutathione peroxidases (GPXs), and catalase (CAT). *See* Figure 2 for a more detailed description of the primary antioxidant mechanisms in living cells.

reactive oxygen species by various lung cells has been reviewed previously (2). Typical sources of oxidant production in inflammatory states, for example in asthma, include the activation of inflammatory cells, activation of nicotinamide adenine dinucleotide phosphate reduced oxidase on cell membranes, and activation of inducible nitric oxide synthase in the cytosolic compartments of cells. Mitochondrial energy metabolism plays a key role in cell survival in situations where oxidant stress is elevated. Oxidant stress enhances the disruption of the mitochondrial electron transport chain, the final effect being either cell injury (necrosis) or programmed cell death (apoptosis). Thus, reactive oxygen species are produced by multiple mechanisms in all cell compartments and play a significant role in the pathogenesis of lung diseases where oxidant stress is increased.

Besides causing cell and DNA toxicity, oxidants participate in the regulation of normal cell homeostasis and modulate signaling pathways associated with cell growth and proliferation (3). Oxidants activate several reactions in the regulation of transcription factors, such as nuclear factor κ B, which then are associated with the induction of antioxidant enzymes in the lung (4). Antioxidant enzymes, by detoxifying reactive oxygen species, constitute the main protective mechanism of lung tissue against free radical–mediated injury. Besides the basic oxidant defense offered by the antioxidant enzymes, individual variability in the levels of these enzymes has been shown to play a role in the development of oxidant-related lung diseases.

General Antioxidant Defense of the Lung

Superoxide dismutases (SODs) are the only enzymatic system decomposing superoxide radicals to H_2O_2 and are hypothesized to play a significant role against oxidant stress, especially in the lung (2, 5). There are three different mammalian SODs: intracellular copper–zinc SOD (CuZnSOD), mitochondrial manganese SOD (MnSOD) and extracellular SOD (ECSOD) (6–8). SODs have been detected in all classes of lung cells but show significant variability in cell-specific localization and expression.

Numerous enzymatic mechanisms participate in H_2O_2 degradation in the lung (Figure 2). The most important H_2O_2 scavenging enzymes include catalase and the glutathione peroxidases, the latter ones being closely associated with the maintenance of reduced glutathione by glutathione reductase and glutathione synthesis by γ-glutamyl cysteine synthase (glutamate cysteine ligase) and glutathione synthase (1, 9). A typical feature in the antioxidant defense of human lung is the high glutathione content in the epithelial lining fluid (~140 times higher than in the circulating blood) (10), and based on this, glutathione and enzymes associated with its maintenance have been suggested to constitute one of the basic antioxidant defense mechanisms of human lung. Various lung cells, however, differ profoundly in their resistance to oxidant stress that is at least partly associated with the cell-specific expression of antioxidant enzymes in the cells. For example, alveolar type II epithelial cells highly express CuZnSOD, MnSOD, and catalase (2) and are resistant to oxidant stress. In contrast, alveolar type I epithelial cells have low expression of antioxidant enzymes and are sensitive to injury and death under conditions of enhanced oxidative stress. Alveolar macrophages have high expression of catalase and consume exogenous H_2O_2 mainly by catalase (11, 12). In addition to these "classic"

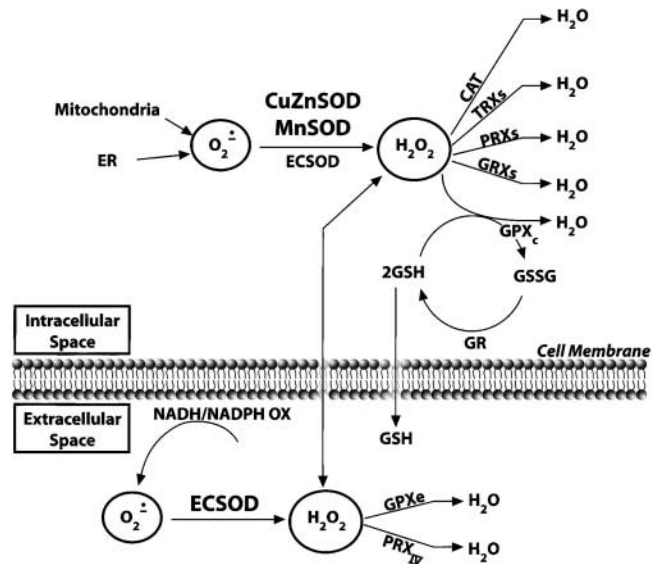

Figure 2. Major antioxidant pathways in the lung. Antioxidant pathways in scavenging superoxide and hydrogen peroxide (H_2O_2) in the intracellular and extracellular spaces. Note: GSH is transported outside the cells by various mechanisms, but the cell membrane is not usually permeable for GSH diffusion. H_2O_2 readily crosses the cell membrane but superoxide does not. In the extracellular spaces, the major source of superoxide is from nicotinamide adenine dinucleotide reduced (NADH)/ nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidases on cell membranes and there are specific extracellular antioxidant enzymes including ECSOD, GPXe, and peroxiredoxin IV. CAT = catalase; ECSOD = extracellular superoxide dismutase; GPXc = classic (intracellular) glutathione peroxidase; GPXe = extracellular glutathione peroxidase; GR = glutathione reductase; GRXs = glutaredoxins; GSH = reduced glutathione; GSSG = oxidized glutathione; PRXs = peroxiredoxins (thioredoxin peroxidase); TRXs = thioredoxins.

antioxidant enzymes, human lung has recently been shown to express several other enzymes with H_2O_2 consuming capacity. These include the thioredoxin–thioredoxin reductase system, thioredoxin peroxidases (peroxiredoxins), and glutaredoxins (13–15). The expression of most of these enzymes is concentrated in airways and in alveolar macrophages of human lung (16, 17). Several reviews have been published on the H_2O_2 scavenging enzymes, such as the reduced glutathione–related mechanisms and catalase in the lung (2, 9, 18).

There are also a number of nonenzymatic antioxidants that are important in maintaining lung redox balance but that are outside the scope of this review. For example, lung extracellular fluids are rich in glutathione, and surfactant proteins such as surfactant protein D have been shown to have a significant antioxidant function. Metals such as iron and copper ions are powerful promoters of oxidative damage, but they are mainly bound to proteins (transferrin, ferritin, ceruloplasmin, lactoferrin, etc.). Several other proteins, for example albumin, have been found to possess efficient free radical scavenging capacity. Other antioxidant defenses include numerous small molecular weight vitamins, either lipid (vitamin E) or water (vitamin C) soluble. The present review concentrates on SODs in the lung with special emphasis on those lung diseases with a likely free radical–related pathogenesis.

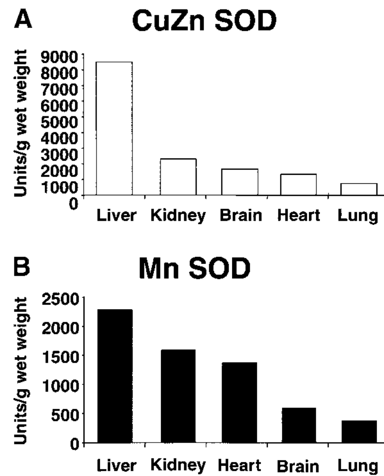

Figure 3. The activities of copper–zinc superoxide dismutase (CuZnSOD) and manganese superoxide dismutase (MnSOD) in selected human tissues. The values for (*A*) and (*B*) were taken from the only available survey where SOD activities have been compared in various human tissues (20) and are from a very small number of samples (n = 2). SOD activity was determined using a potassium superoxide assay at pH 10.0. This assay results in an approximate 10-fold increase in activity for the CuZnSOD (and extracellular superoxide dismutase [ECSOD]) relative to assays done at a physiologic pH. Because MnSOD activity is not proportionately affected by the high pH, the results shown in (*A*) have been reduced by a factor of 10 from that reported by Marklund to express the data at a biologically relevant pH and keep the CuZnSOD activity on a scale that is comparable to the MnSOD.

SODs and Their Distribution in the Lung

Some of the basic characteristics of SODs are given in Table 1. Given the importance of SODs as primary enzymes against superoxide radicals, these enzymes may play a major role not only in the primary defense of human lung against free radicals produced as part of normal metabolism but also are critical in protecting against the progression of oxidant-related lung diseases. SODs not only constitute the basic superoxide consuming mechanism in oxidant-exposed cells, but they also participate in the regulation of normal cell homeostasis. Superoxide radicals are produced continuously as a byproduct of cellular metabolism, being then converted to H_2O_2 (signaling molecule at low concentrations) by SODs. Superoxide is also known to react with nitric oxide, participating in the regulation of vascular tone in normal situations and in the generation of toxic nitrogen metabolites (such as peroxynitrite) in inflammatory states (19). Thus, SODs have multiple functions in regulating intracellular and extracellular levels of superoxide, H_2O_2, and nitrogen metabolites.

When analyzed from total lung homogenates CuZnSOD and MnSOD activities have been reported to be lower in the lung than in several other vital organs such as liver, kidney, heart, and brain (Figure 3) (20). In contrast, ECSOD activity has been reported to be remarkably higher in the lung than in the previously listed vital organs (Figure 4). A few other tissues show even higher levels of ECSOD than that of lung, including thyroid and uterus (20), but among the major solid organs, lung is unique in its high expression of ECSOD. Given the complex architecture and existence of multiple cell types in the lung, *in situ* hybridization, immunohistochemistry, and immunoelectron microscopy in combination with morphometric analysis (21, 22) have given important information about the significance of these enzymes in various cell types and cell compartments of the lung.

By immunohistochemistry and/or immunoelectron microscopy using the immunogold technique, CuZnSOD has been shown to be highly expressed in bronchial epithelium, alveolar epithelium, mesenchymal cells, fibroblasts, arterioles, and capillary endothelial cells in rat lung (23, 24). In the bronchial epithelium, CuZnSOD is expressed most prominently in ciliated epithelial cells (25–27) (Figures 5A and 5B). The subcellular distribution of CuZnSOD has been studied extensively in liver cells, which contain the highest CuZnSOD activity among all tissues investigated (28). CuZnSOD is ultrastructurally located mainly in the cytosol but is also expressed in the nucleus at levels that are 50 to 60% of that of the cytosol and is very highly expressed in some organelles such as lysosomes (23, 28–31).

In situ hybridization studies on MnSOD have revealed a consistent pattern in rat lung messenger (mRNA) distribution with the most prominent labeling occurring in the airways, especially in the septal tips of alveolar ducts and arterioles near the airways (32, 33). Immunohistochemical studies have shown that MnSOD is moderately or highly expressed in respiratory epithelium, alveolar type II epithelial cells, alveolar macrophages, interstitial fibroblasts, and visceral pleura of hyperoxia-exposed rats (23, 24). In healthy human lung, MnSOD is expressed in proportion to the mitochondrial content of cells with cells such as bronchial epithelium, alveolar type II epithelial cells, and alveolar macrophages having the highest MnSOD expression (11, 26,

TABLE 1. SELECTED CHARACTERISTICS OF MAMMALIAN SUPEROXIDE DISMUTASES

SOD	Structure	Metals	Molecular Weight (*kDa*)	Chromosomal Localization	References
CuZnSOD	Homodimer	Cu and Zn	32	21q22	7, 310, 311
MnSOD	Homotetramer	Mn	86–88	6q25	77, 312, 313
ECSOD	Homotetramer	Cu and Zn	135	4q21	6, 38, 42, 120, 315

Definition of abbreviations: CuZnSOD = copper–zinc superoxide dismutase; ECSOD = extracellular superoxide dismutase; MnSOD = manganese superoxide dismutase; SOD = superoxide dismutase.

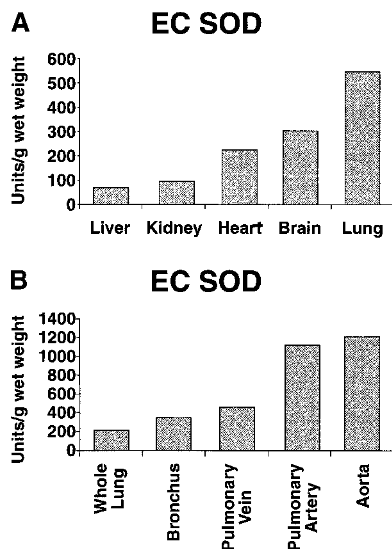

Figure 4. Extracellular superoxide dismutase (ECSOD) activity in the lung. (*A*) The activity of ECSOD in selected human solid organs taken from the survey by Marklund (20). (*B*) Shows the activity of ECSOD in human vasculature, pulmonary vessels, and lung as described by Oury and coworkers (41). The absolute numbers differ from (*A*) for whole lung in part because a different assay was used (cytochrome c at pH 10.0). Because the assay was done at pH 10.0, the values were reduced by a factor of 10 to facilitate comparisons with Figures 1A, 1B, and 2A. ECSOD activity in the lung is shown to be highly concentrated in the airways and in the pulmonary vasculature.

27, 34–36) (Figures 5C and 5D). MnSOD decreases in cultured cells when assessed by the immunogold technique and is remarkably lower (~65%) in SV40 immortalized human bronchial BEAS 2B cells compared with primary cultured cells (26). MnSOD is exclusively localized to the mitochondria (8, 23), a critical cell organelle both in the cellular energy metabolism and cell survival. Overall, MnSOD is most highly expressed in alveolar type II epithelial cells and alveolar macrophages, cell types with relatively high metabolic capacity, and plays a major role in protecting lung tissue against free radicals.

The lung is one of the major tissues that strongly expresses the mRNA and protein of ECSOD (37–41). *In situ* hybridization studies on mouse and human lung have found strong signals of ECSOD synthesis in bronchial epithelium, alveolar epithelium, epithelial cells lining intrapulmonary airways, alveolar macrophages, and endothelial cells lining both arteries and veins. (38, 42). Immunohistochemical studies have revealed that ECSOD is primarily located in the extracellular matrix, but it is also expressed in airway epithelial cell junctions and around the surface of vascular and airway smooth muscle cells (19, 40) (Figures 5E and 5F). ECSOD is the main SOD in pulmonary and systemic vessels (over 70% of the total SOD activity in some vessels) where it is located beneath the endothelium, surrounding smooth muscle cells, and throughout the adventitia of vessels (19, 20, 41) as shown in Figure 4. ECSOD has an affinity for negatively charged molecules such as heparin and the negatively charged molecules in the matrix (43). In the lung interstitium, ECSOD has been shown to be highly localized in areas containing high amounts of type I collagen fibers. The distribution of ECSOD in the interstitial space suggests that it has an important role in protecting matrix proteins against extracellular oxidants, as is the case, for instance, in inflammatory states. A summary of the expressions of MnSOD, CuZnSOD, and ECSOD in normal lung is shown in Table 2.

It is difficult to directly compare the activities of the three SODs in lung because they are localized in compartments of different sizes and have been evaluated by multiple investigators using assays of different sensitivities and specificities and in which all three enzymes were never simultaneously evaluated. Figure 6 attempts to resolve this by correcting activity assays for known impacts of pH on relative activity of the CuZnSODs and MnSOD and by using morphometrically derived data on the volumes of the primary compartments in which the enzymes are distributed. The assumptions that the enzymes are only found in one of the compartments and are uniformly distributed in that compartment are not absolutely correct. For example, CuZnSOD is found in mitochondria and has very high expression in lysosomes, and ECSOD has a minor distribution within cells. Overall, the assumptions are reasonable and the minor exceptions as noted previously would not substantially change the comparisons shown in Figure 6B. As shown in Figure 6A, the dominant SOD in lung is the CuZnSOD with MnSOD and ECSOD each representing ~15 to 20% of the SOD activity. Although CuZnSOD is present in lung in lower concentrations than in other tissues (such as liver and kidney) and ECSOD is higher in the lung than in these other tissues, CuZnSOD is still the dominant lung SOD. SOD concentrations are believed to be closely related to the redox status of specific cell compartments. The comparison of lung with other organs suggests that cytoplasmic production of superoxide is generally lower in lung cells than in highly metabolic cells such as those of liver and kidney, thus requiring lower overall CuZnSOD concentrations. The relatively high ECSOD levels in lung likely relate to the dense airway and vascular network in the lung and the higher potential of extracellular inflammatory events resulting from direct exposure of the lung to the external environment.

Figure 6B illustrates two important concepts. MnSOD is the most highly concentrated SOD within its compartment. Mitochondria are known to be the largest source of superoxide production within cells (44) and, as would be expected, require a very high SOD level to maintain homeostasis. ECSOD, in contrast, is distributed in a much larger compartment and, even with its relatively high lung localization, ECSOD is not present in a sufficient concentration to be able to function as a bulk scavenger of superoxide across the entire extracellular space. This enzyme is unique in that it has a positively charged carboxyterminus that leads to binding in specific domains within the extracellular spaces. The localization of ECSOD rather than its average concentration likely determines its major biological impact. Whereas CuZnSOD and MnSOD are generally believed to act as bulk scavengers of superoxide in the cell cytosol and in mitochondria, ECSOD has been postulated to be more involved in redox-mediated signal transduction, particularly in the regulation of nitric oxide–mediated signaling across extracellular spaces (45).

Expression of SODs during Lung Development

Lung cells are exposed to a sudden severalfold increase in oxygen concentration at birth. In addition, newborn premature infants may be ventilated using high oxygen concentrations that would increase the risk of toxicity to lung cells. Developmental expression of antioxidant enzymes is critically important in these events and in the resistance of newborn lung to high oxygen tensions. Studies on human lung have concluded that CuZnSOD mRNA increases toward adulthood (46), but the protein expression and enzymatic activity of CuZnSOD are similar in prematurely born human infants and adults (46–49). Human MnSOD mRNA increases toward adulthood, but—as with CuZnSOD—the specific activity of MnSOD is similar in neonatal and adult lung (46). Some studies have suggested that MnSOD increases gradually in the peripheral airways, interstitium, and alveolar macrophages during development, but in these studies the immunoreactivity of MnSOD in the bronchiolar epithelium and cuboidal alveolar epithelium (pre–type II pneumocyte cells) was already positive during Weeks 15 to 17 of gestation (47, 49, 50). ECSOD can be detected in the arterial intima, media, bronchiolar epithelium,

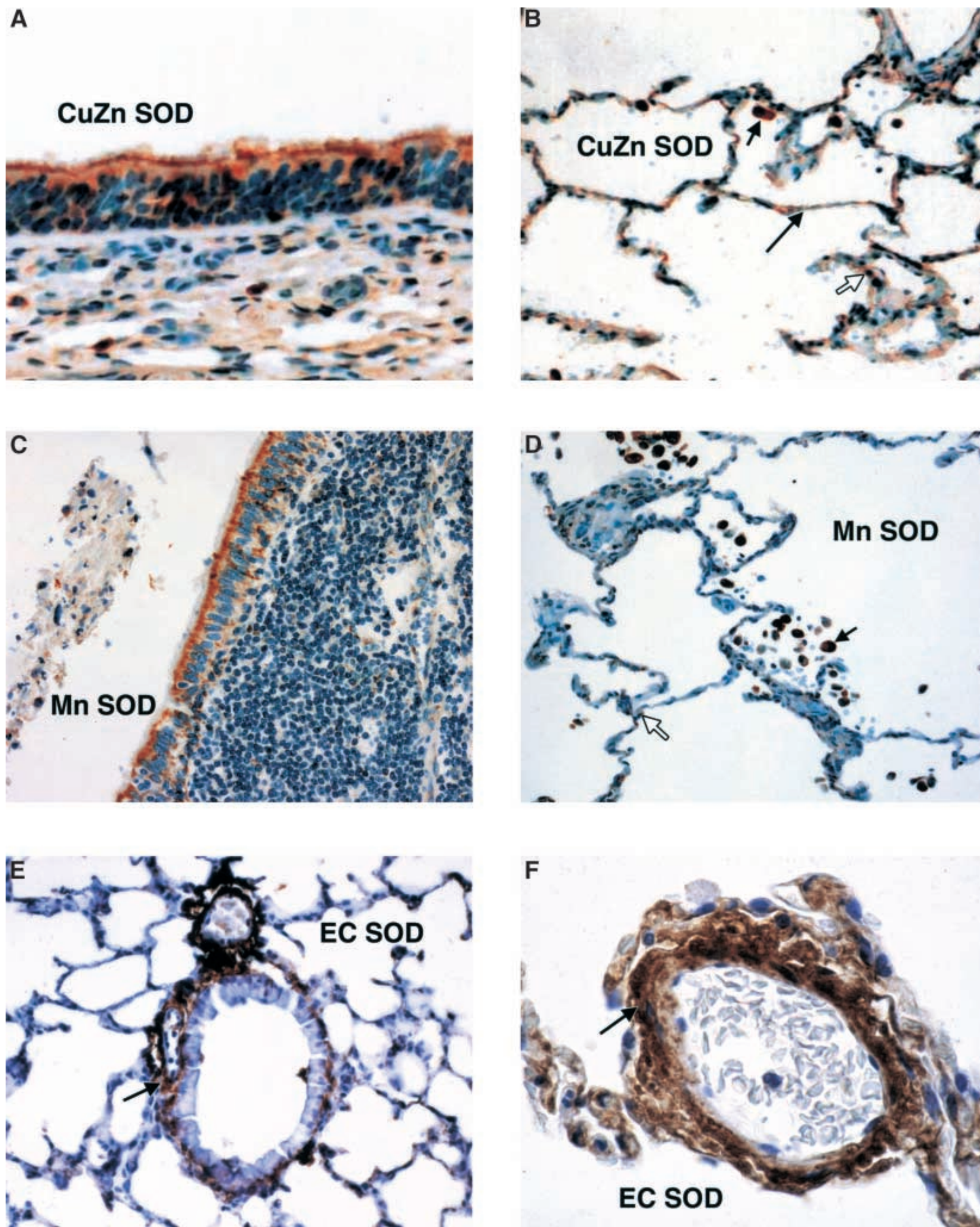

Figure 5. Pulmonary distribution of the superoxide dismutases. Histochemical localization of the superoxide dismutases in human lung using rabbit anti-human copper–zinc superoxide dismutase (*CuZn-SOD*), manganese superoxide dismutase (*MnSOD*), or extracellular superoxide dismutase (*ECSOD*) antibodies. (*A*, *B*) *CuZnSOD* labeling in an airway and in the alveolar region. The enzyme is found in a substantial concentration in the cytoplasm and nucleus of all cells in a relatively homogeneous pattern. Significant uniform labeling for *CuZnSOD* is found in the bronchial epithelium and bronchial walls (*A*) and throughout the alveolar septa (*B*). Labeling can be seen even along the thin cytoplasmic extensions of cells that make up the alveolar septa (*long arrows*). Tissue samples are from smokers who had lung resected for a lung carcinoma. Significant numbers of macrophages are present and show strong labeling for *CuZnSOD* (*short arrows*), cuboidal cells along the alveolar septa label strongly (*open arrow*), and likely correlate with the high *CuZnSOD* activity known to be present in alveolar type II epithelial cells (23). (*C*, *D*) *MnSOD* labeling in a human (smoker) airway wall and in the alveolar region. Again all cells show labeling for *MnSOD*; however, the pattern is more granular and at the electron microscopy level can be shown to correspond to labeling only over mitochondria (23). In (*D*), strong labeling of macrophages (*short arrows*) is present as well as intense labeling of cuboidal cells in the septa (*open arrows*) (102). The septal cuboidal cells likely correspond to alveolar type II epithelial cells that are known to be rich in mitochondria (45). The thin portions of the alveolar septa show minimal labeling for *MnSOD* as would be expected for the long cytoplasmic extensions of type I epithelial cells and endothelial cells that have few mitochondria (310). (*E*, *F*) *ECSOD* labeling of a small airway (*E*) and small pulmonary vessel (*F*). Strong labeling is seen in the airway and vessel walls (*arrows*) corresponding to the activity distribution shown in Figure 4B. By electron microscopic immunocytochemistry, a majority of *ECSOD* labeling can be shown to be extracellular and to have high affinity for matrix elements like collagen (41). A small but distinct *ECSOD* activity can be found intracellularly by electron microscopic immunocytochemistry.

alveolar epithelium, and extracellular matrix of human neonatal lung (51). Overall, both premature and mature human lungs appear to be prepared for the high oxygen tension (21% oxygen) that will be experienced at birth.

There may be significant differences in the developmental profile of SODs in human and animal lungs. Experimental studies on rats, rabbits, guinea pigs, and lambs have suggested that antioxidant enzymes increase during gestation and at birth in most species (52–60). Part of these differences between human and animal lung may be related to the redox-sensitive mRNA-binding proteins that possibly participate in the enzyme regulation during ontogenesis. These proteins can be developmentally regulated so that adult rat lung, for example, contains lower MnSOD RNA–binding activity than neonatal lung (53, 61). In rabbit lungs, ECSOD distribution and activity increase toward adulthood. This may be partly related to changes in the intersubunit disulphide bonds during lung development (62). The localization of ECSOD also appears to be mainly intracellular in

TABLE 2. SUMMARY OF THE MAJOR SITES OF CELLULAR EXPRESSION OF SUPEROXIDE DISMUTASES IN HUMAN LUNG

MnSOD	Alveolar type II epithelial cells
	Alveolar macrophages
	Bronchial epithelium
CuZnSOD	
	Bronchial epithelium
	Alveolar macrophages
	Alveolar type II epithelial cells
ECSOD	
	Airway walls
	Pulmonary vasculature
	Alveolar macrophages
	Interstitium (association with collagen fibers)

Definition of abbreviations: CuZnSOD = copper–zinc superoxide dismutase; ECSOD = extracellular superoxide dismutase; MnSOD = manganese superoxide dismutase.

rabbit fetuses but increases in the extracellular space toward adulthood (63). Thus, there may be species differences in the developmental profile of SODs. Further studies need to be directed toward better understanding of the developmental expression of antioxidant enzymes, especially in human lung.

REGULATION OF SODs

CuZnSOD

CuZnSOD gene contains regulatory elements such as nuclear factor-1, specificity protein-1, activator protein-1 and -2, glucocorticoid response element, heat shock transcription factor, nuclear factor κ B, and the metal responsive element (64, 65). In agreement, the promoter of CuZnSOD has been shown to be induced by oxidants and metal ions (65) and the synthesis of CuZnSOD to increase by heat shock in rat lungs (66) and by shear stress in aortic endothelial cells (67). However, in most studies, lung CuZnSOD mRNA or activity is not induced by cytokines or oxidant stress *in vivo* or *in vitro* (2, 23, 27, 36, 68–73). Cultured endothelial cells exposed to hyperoxia have shown increased CuZnSOD mRNA with the intensive S growth phase of cells, but again there is no increase in enzyme activity (74). Elevated CuZnSOD activity in isolated alveolar type II epithelial cells after exposure of rats to hyperoxia has been found to be primarily associated with hypertrophy of the cells rather than being due to enzyme induction (75). If anything, immunohistochemical staining of CuZnSOD in the bronchial epithelium of ozone-exposed rats has shown lower reactivity than control lungs (76). It appears that CuZnSOD can be induced by certain stimuli, but in the lung, mitochondrial MnSOD—as will be described later—not CuZnSOD is the primary SOD that is substantially induced by hyperoxia and cytokines.

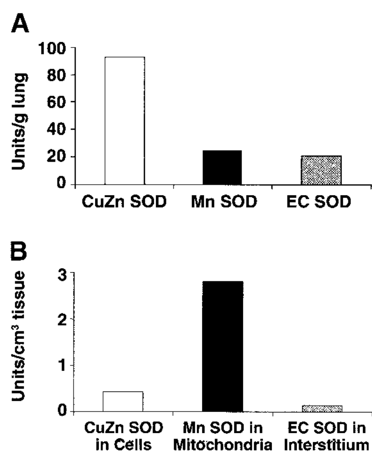

Figure 6. Comparison of copper–zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), and extracellular superoxide dismutase (ECSOD) activities in the lung, derived from Oury and coworkers (41). (*A*) This distribution was calculated from the reports of total cellular SOD activity by Oury and coworkers (19) using a pH 10.0 assay and assuming that the cellular SODs are approximately 80% CuZnSOD and 20% MnSOD, as has been reported for multiple tissues including lung (125). The CuZnSOD and ECSOD data arereduced by a factor of 10 so that comparisons of all three enzymes can be done at a physiologic pH. (*B*) Shows the average concentration of the three SODs in the compartments where they are primarily located. This figure assumes that each enzyme is located in one major compartment and that it is uniform within that compartment. The volumes of the three compartments of interest for normal human lung are (*1*) cellular compartment (CuZnSOD) 216 cm^3, (*2*) mitochondria (MnSOD) 8.6 cm^3, and (*3*) extracellular compartment (ECSOD) 157 cm^3. These data were calculated from Stone and coworkers (21, 22) and the calculations of the mitochondria volume (4%) from the study of Chang and coworkers (23).

MnSOD

The MnSOD promoter contains binding sites for several transcription factors such as activator protein-1 and -2, specificity protein-1, and nuclear factor κ B (77–79). Accordingly, MnSOD mRNA, protein and/or activity are increased in lung homogenates and in cultured pulmonary epithelial cells, endothelial cells, fibroblasts, alveolar, macrophages, granulocytes, and in malignant lung cells by oxidants, cigarette smoke, asbestos fibers, ozone, tumor necrosis factor α (TNFα), IFNγ, interleukins-1 and -6, endotoxin, redox regulators, and during exposure to drugs and thiol-reducing agents (33, 70, 72, 73, 76, 80–90). Cytokine-induced MnSOD activation is rapid and sensitive, commonly requiring only 0.1 to 1 ng/ml concentrations of the cytokine. MnSOD induction can be seen as early as 2 hours, the maximum induction being observed at 24 to 48 hours (72). TNFα, which is the most widely investigated cytokine, increases MnSOD activity and mRNA expression in a dose- and time-dependent manner. The increase at the level of specific activity is 520% in A549 lung epithelial cells after exposure to TNFα (10 ng/ml) for 48 hours, the mRNA level increasing 20-fold after the 48-hour incubation (87, 91). Induction of MnSOD is different in various phases of cell growth, with the highest expression being observed in confluent cells (72). It has been postulated that MnSOD is induced only in nonmalignant cells (92), but at least TNFα leads to MnSOD induction both at the level of mRNA and specific activity in malignant lung adenocarcinoma and pleural mesothelioma cell lines (87, 91). MnSOD mRNA levels do not necessarily correlate with protein expression or enzyme activity, one possible explanation has been suggested to be the MnSOD RNA–binding protein which regulates MnSOD translation (93, 94). As described previously, oxidants and cytokines generally cause MnSOD induction. However, at least peroxynitrite (95–98) and dexamethasone (99) appear to inactivate MnSOD. Peroxynitrite-related MnSOD inactivation has been suggested to be related to the phosphorylation of SOD binding proteins and to the induction of dityrosine formation and tyrosine oxidation in MnSOD (95, 96, 100). Taken together MnSOD is highly inducible by oxidants and cytokines but is inactivated at least by nitrosative stress *in vitro*.

The majority of studies on the regulation of MnSOD *in vivo* have been conducted with animals exposed to high oxygen tensions (hyperoxia). These studies have given valuable information about the regulation of MnSOD both in lung tissue and in individual cells. Exposure of rats to hyperoxia leads to a transient increase in the mRNA of MnSOD (340% maximally) in lung homogenates (69, 101). By *in situ* hybridization, MnSOD mRNA can be detected in arterioles, septal tips of alveolar ducts, endothelial cells, and in pleural mesothelium of hyperoxia-exposed rats (32). MnSOD mRNA obtained from hyperoxic lungs shows increased transcription and message stability (68). One to two

weeks of exposure to hyperoxia also leads to elevated MnSOD immunoreactivity especially in alveolar type II cells and in interstitial fibroblasts (23, 102). Animal models where repeated exposures have resulted in increased tolerance to hyperoxia show increased MnSOD activity, increased MnSOD protein synthesis, and high translational efficiency (94, 103). Acute exposure to severe hyperoxia does not lead to MnSOD induction in human bronchial epithelium (25). MnSOD has also been observed to be downregulated in severe hyperoxia after a transient MnSOD mRNA elevation, and decreased enzyme activity appears to be related to impairment of the MnSOD translational efficiency (68, 94). The studies described previously with hyperoxia-exposed animals and hyperoxia and/or cytokine-exposed cultured cells (2, 36, 71, 104) suggest the importance of cytokines in MnSOD induction. Overall MnSOD is induced significantly by sublethal hyperoxia, whereas it is usually unchanged or downregulated in severe hyperoxia *in vivo*.

Acute hypoxia in hypoperfused or nonventilated rabbit lung leads to significantly decreased (32%) MnSOD activity (105, 106). Rats exposed to hypoxia for 7 days develop tolerance to hyperoxia with elevated MnSOD protein and activity (180%) (107). When cultured alveolar type II epithelial cells and lung fibroblasts have been exposed *in vitro* to air (control cells) or to 5% oxygen (hypoxia) MnSOD mRNA expression has decreased in both types of cells, the decrease being of the order of 20% (108, 109). In agreement, culture of alveolar macrophages under hypoxic conditions has resulted in decreased MnSOD activity (110). The variable results of these studies may be partly associated with the experimental conditions, severity of hypoxia, durations of the exposure, or species investigated. Severe hypoxia, which is often associated with chronic pulmonary diseases, can possibly enhance the inactivation of the most important antioxidant enzymes, such as SODs in the lung, thus predisposing these already injured lungs to further oxidant stress.

ECSOD

The gene for ECSOD contains several regulatory sequences such as activator protein-1, glucocorticoid response element, antioxidant response element, xenobiotic response element, and multiple binding sites for the Ets family of transcription factors (37, 63, 111), but direct oxidant stress does not affect ECSOD expression to the extent it commonly induces MnSOD activity (19). ECSOD, however, is induced by cytokines—at least by TNFα and IFNγ in rat type II pneumocyte (L2) cells (112), by TNFα and IFNγ in fibroblasts (113) and by IFNγ and interleukin-4 in vascular smooth muscle cells (114). The induction of the mRNA for ECSOD occurs within the first hours of oxidant exposure, with ECSOD protein levels rising within 24 hours. ECSOD is downregulated by transforming growth factor-β and interleukin-1 in cultured fibroblasts (113) and by TNFα, epidermal growth factor, platelet-derived growth factor, and granulocyte monocyte-colony stimulating factor in vascular smooth muscle cells (114), suggesting a possibly impaired antioxidant defense in various pathologic conditions *in vivo* where these cytokines and growth factors are expressed.

ECSOD makes up a significant component of the total SOD activity in the lung (20, 41). In one study, neither the distribution nor expression of ECSOD was changed after exposure of rats to hyperoxia (115), whereas exposure to severe hyperoxia resulted in a significant decrease of ECSOD in the lungs and broncholveolar lavage fluid of mice (116). ECSOD appears to be transiently induced in bleomycin-induced acute lung inflammation (117, 118). Exposure of rats to LPS did not reveal major changes in the distribution of ECSOD mRNA in the lung, but high transient ECSOD expression in invading neutrophils and macrophages was suggested to be associated with the defense of inflammatory cells against superoxide radicals (115). An ECSOD molecule contains two dimers with disulphide bonds linking the heparin-binding domains together (119, 120). One feature in the regulation of ECSOD in tissues is its proteolytic cleavage that leads to the removal of the carboxyterminal heparin–binding domain of ECSOD (119, 121) and thereby loss of the enzyme from the extracellular matrix. This cleavage may function as an additional regulatory mechanism of ECSOD *in vivo* because the proteolyzed ECSOD protein has been detected in the bronchial lavage fluid of bleomycin-treated mice (117). In summary, the expression of ECSOD is elevated to some extent during acute inflammation of the lung. Proteolytic removal of the heparin-binding domain of ECSOD has been suggested to be an important regulatory mechanism in the distribution of ECSOD within the extracellular space.

Induction of SODs and Tissue Protection

Exposure to sufficient hyperoxia or cytokines to cause MnSOD induction has been associated with increased tolerance to hyperoxia. Animals and/or cells pre-exposed to sublethal hyperoxia or TNFα are more resistant to subsequent exposures, and this resistance correlates with elevated SOD activity (5, 92, 122, 123). The association between SOD activity in the lung and oxidant protection, however, is not necessarily causal as has also been emphasized. Tolerance to hyperoxia is not related only to changes in the SODs because in these circumstances the lungs undergo multiple adaptive phenomena and the levels of several antioxidant enzymes (e.g., catalase, glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase) are simultaneously elevated (69, 124–127). Protection can also be associated with the severity of the exposures and may be species and cell specific (94, 128). Moreover, oxidant protection has been suggested to be related to mechanisms other than antioxidant enzymes (36, 129). Thus, lung protection against oxidant stress is complex, and several mechanisms in addition to the SODs are involved. (130).

GENE MANIPULATION AND LUNG PROTECTION

CuZnSOD

Numerous *in vitro* studies with CuZnSOD have been conducted with neurons due to the importance of CuZnSOD in degenerative neurologic disorders (131, 132). Transfection of CuZnSOD into the cytosol of cultured cells has shown that protection is not dramatic and that overexpression of CuZnSOD without an increase in H_2O_2 scavenging mechanisms can be harmful. Simultaneous increases in CuZnSOD and in H_2O_2-decomposing antioxidant enzymes appear to give better protection than induction of CuZnSOD alone, and isolated CuZnSOD overexpression can enhance cell senescence and apoptosis (133). Shortened survival in cells with isolated CuZnSOD overexpression has been suggested to be due to an increased H_2O_2 level in the cells (133, 134). The experimental evidence for the elevated H_2O_2 levels in SOD overexpressing cells with consequent toxicity, however, is controversial. Liochev and Fridovich (135) have suggested that due to competitive reactions SOD overexpression may in fact decrease the overall H_2O_2 production. In agreement, recent mathematic assessments concluded that SOD overexpression may decrease, increase, or have no effect on H_2O_2 production, the outcome depending on the ratio between the rates of superoxide reduction and the rates of the non-H_2O_2–producing reactions at normal levels of SOD expression (136). Thus, the final effects of SOD overexpression on oxidant status and cell protection are complicated and depend on the oxidant and antioxidant reactions of the cell.

Studies with transgenic mice overexpressing CuZnSOD have not shown significant lung protection from hyperoxia (137, 138) or from experimentally induced endotoxemia (139). CuZnSOD may, however, play a role in other stress conditions of the lung. Mice overexpressing CuZnSOD were, for example, resistant to allergen-induced lung toxicity (140). Young transgenic mice overexpressing CuZnSOD were also more resistant to high oxygen tensions than their control littermates, but these mice also had elevated catalase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase activities (141). Overall, the balance of several antioxidant enzymes appears to be more important than the level of isolated CuZnSOD in lung protection against high oxygen tension.

The studies with CuZnSOD knockout mice have not found any significant changes in the lungs or other vital organs (142). Fetal fibroblasts derived from mouse fetuses deficient in CuZnSOD, however, show increased sensitivity to paraquat, a compound that induces superoxide generation mainly in the cytoplasmic compartment (143). These studies with knockout animals and cultured cells confirm previous suggestions that CuZnSOD has major importance in the cytosolic compartment. Evidently, constitutive levels of cytosolic CuZnSOD have a role in lung protection, but protection cannot be substantially improved by genetic manipulation of CuZnSOD.

MnSOD

In contrast to CuZnSOD, there are numerous studies showing that transfection of MnSOD protects various cells against oxidants and oxidant-generating compounds. Resistance is increased at least to hyperoxia (144), oxidants such as paraquat (145), cigarette smoke (146, 147), radiation (148, 149), injury caused by asbestos fibers (150), cytokines such as TNFα (148, 151) and interleukin-1 (152), and drugs such as doxorubicin and mitomycin (148). MnSOD can inhibit apoptosis in lung cells (153), mouse liver cells (154), and malignant cells such as fibrosarcoma (155) and hepatocellular carcinoma cells (156) by controlling mitochondrial reactive oxygen species generation. Besides increasing oxidant resistance, overexpression of MnSOD has antiproliferative effects and tumor suppressor characteristics (157, 158). MnSOD modulates DNA-binding activities and transcriptional activation of redox-sensitive transcription factors and oncoproteins (159, 160). As with CuZnSOD, overexpression of multiple antioxidant enzymes can be more beneficial than isolated overexpression of MnSOD alone. MnSOD protects lung cells against hyperoxia, but simultaneous overexpression of H_2O_2 scavenging enzymes such as catalase offers additional protection (161). Antisense technology to decrease MnSOD activity has not been systematically investigated in lung cells, but in several others (e.g., nonmalignant and malignant cells) it results in lowered oxidant resistance and increased sensitivity to TNFα and IFNs α and γ (151, 162, 163). In summary, the major findings of MnSOD transfection to airway epithelial and/or pulmonary endothelial cells are significant protection against several oxidant-producing agents *in vitro* and *in vivo*.

The importance of MnSOD in lung protection has also been proved in transgenic animals. Transgenic animals created using a surfactant protein C promoter and human MnSOD cDNA exhibit an elevated MnSOD level in alveolar type II epithelial cells and bronchiolar epithelial cells (60% higher total lung MnSOD activity and fourfold higher levels of MnSOD immunoreactive protein in the mitochondria of type II cells). These animals survive significantly longer in 95% oxygen compared with nontransgenic littermates (164), suggesting that MnSOD is critical in modulating pulmonary oxygen toxicity in distal respiratory epithelium. Transgenic mice created using the human β-actin promoter to express the human MnSOD widely in the lung (pneumocyte II cells, endothelial cells, fibroblasts, 300% increase in activity) showed no or marginal advantage in survival after exposure to hyperoxia (137, 165). The reasons for the controversies of these two studies remain unclear but may be related to different expression profiles of the MnSOD transgene in various lung cells, disturbance of the ideal antioxidant/oxidant balance, or genetic background of the mice in these models. Compartmentalization plays an important role in oxidant resistance because, for example, introduction of CuZnSOD in MnSOD knockout animals with consequent cytosolic overexpression of CuZnSOD did not prevent oxygen-related neonatal lethality (166). This finding further supports the importance of mitochondria as the major site of reactive oxygen species production and that mitochondria are early targets of oxidant injury. Results with MnSOD transgenic studies suggest the importance of MnSOD in protecting lung tissue against high oxygen tension, provided the enzyme is targeted to the mitochondria of alveolar type II cells.

The total deficiency of MnSOD is lethal, but the lungs in $MnSOD^{-/-}$ mice are only minimally affected. The most important manifestations in MnSOD knockout mice include degenerative changes in large areas of the central nervous system, especially in the basal ganglia, and cardiotoxicity (138, 167, 168). In studies with a deleted exon (168) or deleted exons 1 and 2 (167) of the MnSOD gene, the main findings are similar (i.e., no apparent lung toxicity but increased susceptibility to superoxide-induced mitochondrial damage in the central nervous system and heart). The minimal changes reported to occur in the lungs of $MnSOD^{-/-}$ mice include alveolar dilatation suggestive of delayed postnatal lung development, but due to the severe lung atelectasis more defined assessment of those abnormalities has remained unclear (169). In agreement, exposure of MnSOD knockout animals to 50% oxygen appears to lead to undefined changes in the alveolar general structure of mouse lung (169).

Studies on MnSOD heterozygous mice having an approximate 50% reduction in the MnSOD activity do not reveal sensitization to hyperoxia or pertussis toxin–induced lung injury (170–172). This suggests that at least in mice 50% MnSOD activity is enough for normal oxidant resistance of the lung. MnSOD heterozygous mice have decreased lung glutathione concentration (173), and muscle and cardiac mitochondria show increased oxidative damage and an increased tendency for apoptosis (173, 174). The low lung glutathione levels suggest that decreased MnSOD lead to some degree of oxidative stress in the lungs. Interestingly, aged MnSOD heterozygous mice have increased risk for lung cancer (175) and pulmonary fibrosis (176). The results on MnSOD knockout homozygous and heterozygous animals and cells deficient in MnSOD suggest that MnSOD plays a major role in protecting the most important vital organs, especially brain and heart, against endogenous oxidant stress. Half of the normal MnSOD activity is sufficient to block acute oxidative stress in otherwise normal mouse lungs but may contribute to the development of lung fibrosis and lung cancer.

ECSOD

ECSOD is postulated to be of critical importance in the lung extracellular matrix, including the pulmonary vasculature, by consuming superoxide and thereby diminishing the reaction between nitric oxide and superoxide. ECSOD has been shown to protect lung interstitium against free radicals generated during inflammation (19). A threefold increase of ECSOD in alveolar type II and nonciliated bronchial epithelial cells driven by the specificity protein-C promoter leads to protection against hyperoxia partly by attenuating neutrophilic inflammation (177). In mouse lung, ECSOD has been shown to attenuate inflammation when assessed from the samples of bronchoalveolar lavage (178) and alveolar structure (179). Overexpression of lung ECSOD

also attenuates lung injury after hemorrhage when analyzed by lung wet to dry weight, lipid peroxidation, activation of nuclear factor κ B, and myeloperoxidase activity (180). Transgenic mice with elevated brain ECSOD were more susceptible to hyperbaric oxygen than control animals. This apparently paradoxical response is believed to be due to high ECSOD expression in the cerebral vasculature leading to sparing of nitric oxide in those vessels and thus blocking the normal vasoconstrictive response of the cerebral vasculature to hyperoxia. By preserving brain blood flow, the ECSOD transgenic animals actually have a higher cerebral oxygen delivery and thus greater sensitivity to oxygen-mediated toxicity (181). The functions of ECSOD are cell and tissue specific and associated with both regulation of nitric oxide–mediated intracellular signaling and with preservation of the extracellular oxidant–antioxidant balance.

Mice lacking ECSOD develop normally and remain healthy until at least 14 months of age. However, when exposed to more than 99% oxygen, these mice display shortened survival compared with control animals and develop more severe lung damage, including vascular congestion, septal thickening, and an increased number of neutrophils in the lung (182). These findings suggest that under normal physiologic conditions other antioxidant enzymes can balance for the loss of ECSOD, whereas under conditions of inflammatory stress ECSOD plays a critical role in protecting the lung and reducing the amplification of the injury

Exogenous SODs, SOD Mimetics, and Lung Protection

Numerous studies have investigated whether SOD enzyme proteins given either by injection or inhalation can protect lung tissue against oxidant injury. In most models some protection has been obtained, with intratracheal instillation generally providing better protection than intravenous or intraperitoneal injections. These models have mainly used CuZnSOD and concluded that exogenous CuZnSOD by inhalation or instillation provides significant protection against acute lung injury although conflicting results have also been obtained (183–185). Protection of the lung against oxidant injury has been improved by liposome encapsulation of CuZnSOD, which is postulated to provide a longer half-life and better penetration of the enzyme to the target cells (185, 186). Lecithinized SOD (phosphatidylcholine-SOD) has characteristics similar to liposome-encapsulated SOD, and it has protected against bleomycin-induced pulmonary fibrosis when analyzed by lung hydroxyproline content, neutrophil and lymphocyte accumulation, and expression of several cytokines (187). Efficacy has further been improved when liposomes containing CuZnSOD have included catalase, which decomposes H_2O_2 (185, 186, 188). In agreement, polyethylene glycol–attached CuZnSOD + catalase and/or polyethylene glycol catalase provides better protection against hyperoxia and fiber-induced lung injury than polyethylene glycol SOD alone (189–191). SOD-conjugated polyethylene glycol attenuates airway responsiveness in rabbits, suggesting a role of free radicals also in airway inflammation and hyperreactivity (192). Aerosolized delivery of recombinant human MnSOD is even more efficient, protecting baboon lung against 100% oxygen. It decreases pulmonary $\dot{Q}_S/\dot{Q}_T$, preserves arterial oxygenation, decreases lung edema and, by quantitative morphometry, shows protection of alveolar epithelium from hyperoxia (193, 194). Recombinant human MnSOD is positively charged and, when administered in this form, is likely to behave more like ECSOD (i.e., an ECSOD whose charge leads to affinity to negatively charged extracellular elements such as glycosaminoglycans.). No corresponding studies have been conducted on ECSOD.

Several classes of synthetic SOD mimetics have recently been developed and shown to possess significant antioxidant capacity. These new antioxidants provide an alternative approach to attenuate oxidant-related lung injury *in vivo*. These small molecular weight compounds distribute more easily to tissues, potentially reaching high concentrations in intracellular domains and avoiding the problem of antigenicity that is a concern with antioxidant proteins. The three classes of metal-containing substantial SOD mimetics with SOD activity include the salen compounds (195), macrocyclics (196), and metalloporphyrins (197) (Figure 7). In addition to these compounds, nitroxide SOD mimetics have been shown to have free radical scavenging capacity (198–200). The different forms of SOD mimetics have been found to be efficacious in a variety of *in vitro* and *in vivo* animal models, but they have not yet been tested in humans.

The salen class of antioxidant mimetics shares a three-ring aromatic structure containing Mn (195). The prototype of these compounds is EUK-8, which expresses both SOD and catalase-like activities. Newer versions include EUK-134, EUK-178, and EUK-189 with the newer salen compounds reported to exhibit higher catalase activity (201). EUK-8 has been shown to ameliorate acute lung injury assessed by arterial hypoxia, pulmonary hypertension, and lung malondialdehyde content (an indicator of lipid peroxidation) after 4 hours of LPS exposure. It also prevents manifestations of LPS-induced respiratory distress syndrome in pigs by detoxifying reactive oxygen species without affecting the release of other important proinflammatory mediators (202). Besides lung protection, the salen antioxidant compounds also have protective effects on other organs. They prolong the survival of MnSOD knockout mice, decrease motor neuron degeneration and apoptosis (203–206), attenuate mitochondrial abnormalities (207), and attenuate injury to the kidney, liver, skeletal muscle, pancreas, and heart (208, 209). Overall, EUK compounds have

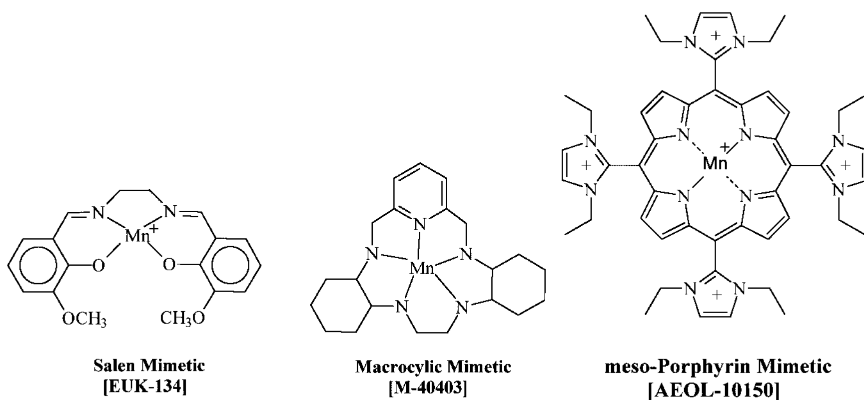

Figure 7. Examples of new classes of metal-containing antioxidant mimetics. Each of these compounds uses manganese as the metal center. The manganese is held by either four or five ligands. The compounds with four ligands have the capacity to do either one or two electron transfers involving a variety of free radicals including superoxide (O_2^-), hydrogen peroxide (H_2O_2), lipid peroxyl (LOO), and peroxynitrite (ONOO–). The macrocylic class of mimetics has five ligands that will limit it to one electron transfers and thus create a more specific mimetic of the SODs. All have much higher free radical scavenging capacity than noncatalytic antioxidants, and all have been shown to be highly effective in a variety of animal models of oxidative stress.

been shown to protect lung and several other organs in experimental oxidant exposures *in vivo*.

The macrocyclic class of antioxidant mimetics binds Mn in a five-ring structure so that the metal is available for single electron transfers. They are believed to be relatively specific scavengers for superoxide (196). A prototypic macrocyclic SOD mimetic, M40403, has been suggested to be especially important in inflammation. It has been potent in carrageenan-induced pleurisy in rats. In this model, it decreases pleural fluid, neutrophil accumulation, and the concentration of several inflammatory cytokines (196). Besides, in the lung, macrocyclics have been found to protect against the inflammatory states of several other organs (210).

The metalloporphyrin class of antioxidant mimetics exhibit strong antioxidant properties, including scavenging of superoxide, H_2O_2, peroxynitrite, and lipid peroxyl radicals (197, 211). They exhibit definite, but low, catalase-like activity that has been attributed to their extensive conjugated ring system and reversible one-electron oxidations. Their capacity to scavenge peroxynitrite apparently involves the formation of an oxo-Mn (IV) complex that can be reduced to the Mn(III) oxidation state by endogenous antioxidants. One of the widely investigated metalloporphyrin antioxidant compounds is Mn(III) meso-tetrakis (4-benzoic acid) porphyrin (212). Mn(III) meso-tetrakis (4-benzoic acid) porphyrin has been shown to protect against paraquat-induced pulmonary endothelial cell injury *in vitro* (211), paraquat-induced lung injury also *in vivo* (213), and carrageenan-induced pleurisy (214). In a rat pleurisy model, Mn(III) meso-tetrakis (4-benzoic acid) porphyrin has decreased leukocyte recruitment, myeloperoxidase activity, histologic lung injury, peroxynitrite formation, and DNA damage. Mn(III) meso-tetrakis (4-benzoic acid) porphyrin has been shown to attenuate the severe lung fibrosis occurring in mice exposed to bleomycin (215). Other closely related metalloporphyrins (MnTE-2-PyP and MnTDE-1, 3-ImP) have been recently shown to protect against cigarette smoke–induced lung injury, antigen-induced broncho-obstruction and development of bronchopulmonary dysplasia (216–220). Besides preventing lung injury, these antioxidants have been found to protect against liver failure (221), zymosan-induced shock (222), cardiomyopathy (223), and neurologic manifestations (224, 225). Overall, new, targeted SOD mimetics have efficient antioxidant capacity and are efficient in preventing lung injury in animal models of enhanced oxidative stress.

Free Radicals in the Pathogenesis of Human Lung Diseases

Free radicals have an evident role in obstructive lung diseases. Asthma is associated with chronic inflammation and an increased oxidant burden. Inflammatory cells of the airways of patients with asthma are activated and generate increased amounts of free radicals. Exhaled air of patients with asthma contains elevated levels of H_2O_2, nitric oxide, and organic volatile compounds (226–230). The bronchial epithelium of patients with asthma shows elevated expression of inducible nitric oxide synthase, which has been linked to increased nitric oxide levels in the exhaled air (230, 231). Thus multiple reactive oxygen species–producing pathways are simultaneously induced in asthmatic airways as shown in Figure 8.

There is evidence that free radicals play a central role in chronic obstructive pulmonary disease (232–234). Chronic obstructive pulmonary disease is related to cigarette smoking in over 90% of cases. One puff of smoke contains 10^{14-16} free radicals (235), and the airways of patients with chronic obstructive pulmonary disease contain increased numbers of neutrophils (234) that, when activated, have a high capacity to produce free radicals. In addition to the direct toxic effects of cigarette smoke and neutrophil-derived free radicals, activated neutrophils inactivate the natural protease system of the lung, the most intensively investigated of them being α-1-antitrypsin. This inactivation enhances the progression of emphysema in the lung.

Free radicals participate in the pathogenesis of numerous interstitial lung diseases. Idiopathic interstitial pneumonias (236, 237), especially usual interstitial pneumonia, represent parenchymal lung diseases with a poor prognosis and histopathologic similarities with hyperoxia-induced lung injury. In contrast to hyperoxic lung injury, usual interstitial pneumonia has patchy lung involvement with a variable stage of fibrosis (active fibrogenesis occurring in the so-called fibroblastic foci) and a low grade of inflammation (238). There are numerous studies showing that the oxidant burden in the lungs of the patients with pulmonary fibrosis is increased and that inflammatory cells of these patients generate more radicals than the cells of healthy control subjects (239–245). In addition, the inflammatory cells of these disorders show elevated inducible nitric oxide synthase expression that then may cause toxicity to multiple cell types of the lung (246, 247). Inflammatory cells of patients with sarcoidosis generate more radicals than the cells of healthy control subjects (248), and the glutathione content of the airways in another granulomatous lung disease—allergic alveolitis—is decreased (249), suggesting increased oxidant burden in granulomatous lung disorders. Furthermore, the granulomas and inflammatory cells of sarcoidosis and allergic alveolitis express elevated levels of inducible nitric oxide synthase as a marker of the oxidant stress in the lung (246). The most widely investigated environmental oxidant-generating agents are asbestos fibers. Asbestos fibers are associated with reactive oxygen species generation both *in vitro* and *in vivo* (250, 251) and induce antioxidant enzymes, most importantly MnSOD in bronchial epithelial and mesothelial cells (252–254). The production of reactive oxygen species caused by asbestos fibers is believed to be part of the pathogenesis of the resulting fibrotic lung disease (asbestosis). Extracellular macromolecules, including collagen, cartilage, pulmonary surfactant, and proteinase inhibitors, are sensitive to superoxide- and peroxynitrite-mediated damage (19, 255), which then may have potential implications for the enhancement of parenchymal lung diseases. One of the most important growth factors in the pathogenesis of fibrotic lung diseases is the transforming growth factor-β. Importantly, latent transforming growth factor-β, which is abundantly expressed in healthy lung, is activated by free radicals (radiation, nitric oxide) (256, 257). Overall, free radicals play a central role in the initiation of fibrogenesis by activating transforming growth factor-β and in the progression of lung injury by activated inflammatory cells.

The high concentration of oxidants in cigarette smoke has been postulated to contribute to carcinogenic impact of cigarette smoke in the causation of lung cancer (258–260). Besides cigarette smoke, asbestos fibers lead to DNA strand breaks, increase cell proliferation, and cause both lung cancer and mesothelioma (253, 261). During these events, cells will carry multiple genetic alterations including inactivation of tumor suppressor genes and activation of oncogenes that will help the escape of the cells from normal growth control. All these events are at least partly regulated by changes in the cellular redox state. The most important of these reactions that have been shown to be regulated by oxidants include mitochondrial apoptosis, apoptosis caused by p53, and growth and senescence regulated by several other oncogenes such as CKIp21 (Cip1) and PTEN (262–266). Recent studies also suggest that hypoxia-associated reactive oxygen species generation at the mitochondrial electron transport chain (ubiquinone) contributes to hypoxia-inducible factor stabilization with consequently increased angiogenesis (267). Thus, the association of reactive oxygen species both in carcinogenesis and in cancer progression has been shown in numerous investigations.

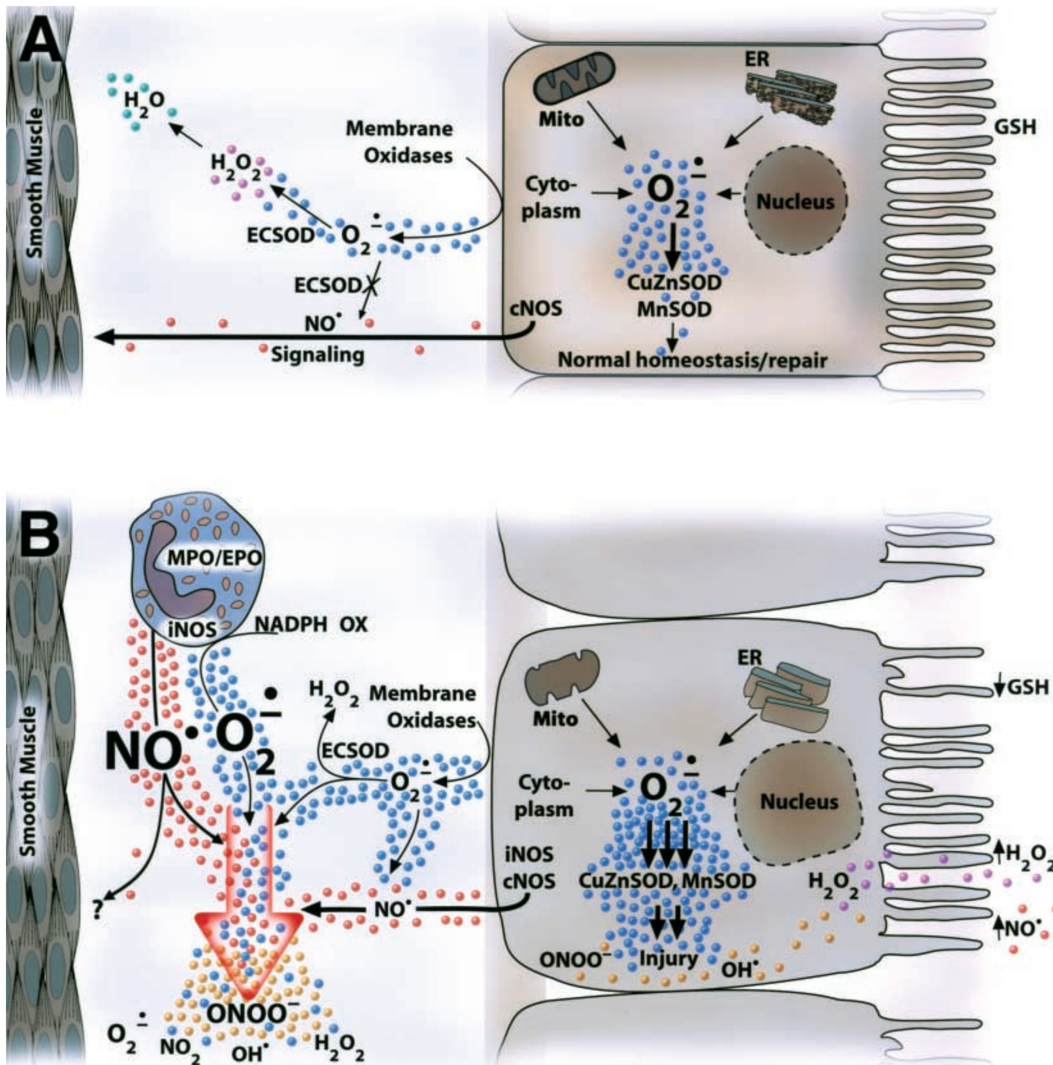

Figure 8. Sources of oxidative stress in lung inflammatory diseases. (*A*) During normal cell homeostasis, superoxide radicals are produced in several cell compartments such as mitochondria, endoplasmic reticulum, cytosol, nuclear membrane, and cell membrane (in both phagocytic and nonphagocytic cells). The intracellular production of superoxide and H_2O_2 is balanced by the high constitutive levels of antioxidant enzymes, and there is little stress or reparative pathways to maintain normal homeostasis. Extracellular production of superoxide is quite low in the absence of inflammation and is balanced by the presence of ECSOD in airway walls. Intracellular nitric oxide (NO·) signaling is protected from superoxide attack by the presence of ECSOD. (*B*) During oxidative stress (e.g., asthmatic inflammation) both superoxide-generating pathways and inducible nitric oxide synthase (iNOS) are induced inside cells. Inflammatory cells such as eosinophils, monocytes, and neutrophils are recruited to the subcellular space beneath the airway epithelium. Nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase on cell membranes, iNOS, myeloperoxidases (MPO) and eosinophil peroxidase (EPO) are activated in the inflammatory cells. Increased oxidant stress leads to disruption of normal homeostasis, and NO·-mediated signal transduction is disrupted with conversion of NO· to proinflammatory and cytotoxic species such as peroxynitrite. No studies have been done to determine if ECSOD levels are modified up or down in inflamed airways.

Free radicals are closely associated with a variety of acute lung diseases. These include oxygen-related lung injury of prematurely born neonates, which can lead to bronchopulmonary dysplasia (chronic lung disease) (268). The use of high oxygen concentrations in the treatment of severe lung complications leads to problems of acute oxygen toxicity in intensive care departments. Furthermore, free radicals have a central role in lung diseases such as infections, pleural disorders, and uncommon pulmonary diseases such as cystic fibrosis, primary pulmonary hypertension, and complications associated with lung transplantation (269–272). Several drugs are associated with reactive oxygen species generation and lung injury with the most widely investigated of them being bleomycin (187). Bleomycin is a chemotherapeutic agent that causes oxidant-mediated lung injury and lung fibrosis both in laboratory animals and in humans (273, 274). Numerous other chemotherapeutic agents, including carmustine (BCNU), antracyclines, antimetabolites, and antibiotics (nitrofurantoin), cause lung injury by reactive oxygen species–mediated mechanisms (1). Overall, free radicals are involved not only in the pathogenesis of lung diseases but also in lung injury caused by drug therapies and radiation.

SOD Polymorphisms and Lung Diseases

SODs are coded by different genes, and their genetic variation plays a role in the pathogenesis of several free radical–associated disorders (131). Over 90 mutations of CuZnSOD have been characterized, many of which are associated with a reduction in specific enzyme activity to 50 to 80% of that of the wild-type protein (131). CuZnSOD polymorphisms are known to be associated with degenerative neurologic diseases (132), but these polymorphisms have not been investigated in human lung diseases.

MnSOD is synthesized in the cytoplasm as a precursor molecule containing the N-terminal leader sequence, which is removed during the transport of the molecule to the mitochondria (77, 275, 276). A polymorphism of MnSOD has been described, where a single nucleotide change in the N-terminal region of the DNA encodes the signal sequence of either alanine (Ala) or valine (Val) in the mitochondrial targeting sequence (276, 277). In a study of over 1,100 patients with lung cancer, individuals with ValVal and ValAla genotypes had a higher risk for lung cancer than the AlaAla genotype (odds ratio 1.67 and 1.4) (278). A similar tendency was observed with mesothelioma (odds ratio

2.0 and 1.4), but the risk did not reach statistical significance, probably due to the small number of the patients in the study (61 cases with mesothelioma) (279). On the basis of recent findings, MnSOD polymorphism was not associated with asthma (Kinnula VL, Lehtonen S, Koistinen P, Kakko S, Savolainen M, Kere J, Ollikainen V, Laitinen T, unpublished). In addition to the polymorphisms described previously, Ho and Crapo have reported the amino acid substitution $Thr^{58}Ile$ in studies on a human cDNA library (280). The Ile-form of MnSOD exhibits threefold higher activity than the Thr-form in transfected MCF-7 cells (281), possibly due to conformational changes in the protein molecule. This polymorphism has not been described in human lung diseases (131, 282). In summary, signal sequence polymorphisms leading to disturbances where essential proteins are not properly targeted, as is the case with MnSOD, have been shown to be associated with the development of malignant lung diseases.

A single nucleotide substitution resulting in an amino acid change from Arg to Gly at 213 (in the middle of the positively charged sequence of the heparin-binding domain of ECSOD) (283) leads to increased circulating ECSOD activity (10-fold). It causes decreased anchoring of ECSOD to negatively charged elements such as heparin in the interstitium and elevated serum levels of ECSOD in those individuals with the decreased heparin-binding variant. Approximately 94 to 98% of the population exhibits the normal (low) level of serum ECSOD activity (283–285). This polymorphism has been described (in three patients) with chronic obstructive lung disease (286). Recent studies, however, have shown that ECSOD polymorphism is not associated with asthma or idiopathic pulmonary fibrosis (Kinnula VL, Lehtonen S, Koistinen P, Kakko S, Savolainen M, Kere J, Ollikainen V, Laitinen T, unpublished). It is likely that the polymorphism of ECSOD, being very rare, cannot explain the risk for the development of common lung diseases. Two additional polymorphisms described in the human ECSOD gene do not have any effects on the heparin-binding capacity or activity of this enzyme (285). These findings do not exclude the possibility that together with other impairments in the patients' oxidant-decomposing mechanisms—genetic or environmental—SOD genes become significant in inflammatory or fibrotic lung diseases where oxidant stress is increased.

SODs IN HUMAN LUNG DISEASES

Obstructive Lung Diseases

Mice overexpressing CuZnSOD are resistant to allergen-induced lung toxicity (140), suggesting that SOD is important in the pathogenesis of allergic inflammation and asthma. In agreement, in most human studies SOD activity decreases in the bronchial epithelium (~50%), in the cells of bronchoalveolar lavage (25%), and in bronchial brushings (nearly 50%) in patients with asthma compared with control subjects (287, 288). Not only the airways of patients with asthma but also blood monocytes and neutrophils of patients with asthma show a lower MnSOD activity than corresponding cells in control subjects (289). Acute antigen instillation into the lungs of individuals with atopic asthma also leads to a rapid 50% decrease in SOD activity in the bronchoalveolar lavage cells of these individuals (287). Because of the abundance of ECSOD in the lung, part of a decrease in SOD activity in asthma may reflect ECSOD activity, although this has never been directly assayed. In summary, oxidant stress is a component of allergic airways diseases, and a decreased capacity to scavenge superoxide, at least in asthma, may exaggerate progression of the disease.

Serum of patients with chronic obstructive pulmonary disease has been reported to contain elevated levels of ECSOD (30%) compared with that of control subjects (286). In that study, three patients with highest levels of serum ECSOD displayed a single base pair guanine to cytosine missense mutation in the coding region of ECSOD at position 213, which is the same polymorphism described previously as leading to decreased anchoring of ECSOD to the interstitium (283). No studies have been reported on the expression of SODs in lung tissue of patients with chronic obstructive pulmonary disease.

Interstitial Lung Diseases

Only a few studies have investigated the expression of SODs in granulomatous diseases and idiopathic pneumonias of human lung. In normal lung, CuZnSOD is mainly localized to bronchial epithelium, and its expression is similar in healthy lung and pulmonary sarcoidosis (27). Unchanged reactivity of CuZnSOD in control subjects and in patients with sarcoidosis is in agreement with several previous notions that CuZnSOD is not induced by cytokines or oxidants in the lung. MnSOD is elevated in alveolar macrophages and in the granulomas associated with pulmonary sarcoidosis and allergic alveolitis (27). MnSOD is also at least moderately expressed by immunohistochemistry in alveolar type II epithelial cells and macrophages in inflammatory areas of usual interstitial pneumonia and desquamative interstitial pneumonia, but its reactivity appears to be low in the late fibrotic lung lesions of usual interstitial pneumonia (35). These human studies confirm previous animal studies and show that MnSOD is induced during acute inflammatory stages of the lung parenchyma and suggest that antioxidant defense (at least MnSOD) is impaired during the progression of fibrogenesis.

Respiratory Distress Syndrome and Bronchopulmonary Dysplasia

CuZnSOD is expressed mainly in bronchial epithelium, and its immunoreactivity is similar or decreased in acute respiratory disorders of neonatal lung compared with healthy neonatal lung (47, 48). MnSOD expression has been reported to be similar in respiratory distress syndrome, bronchopulmonary dysplasia, age-matched control subjects, and healthy human newborns (47, 50) with the exception of intensive MnSOD immunoreactivity in proliferating alveolar type II cells in bronchopulmonary dysplasia (47). This latter finding is in agreement with a report of increased MnSOD levels in the baboon model of bronchopulmonary dysplasia (290). Preliminary studies with respiratory distress syndrome have found that ECSOD is expressed in the arterial intima and endothelium, and in the metaplastic alveolar epithelium, chondrocytes and hyaline membranes of respiratory distress syndrome (51). The expression of ECSOD in the metaplastic epithelium of this disorder suggests the induction of ECSOD and an attempt to increase antioxidant capacity by the diseased lung. In a recent preliminary study, alveolar macrophages, alveolar epithelium, and interstitium of patients with bronchopulmonary dysplasia suggested enhanced ECSOD reactivity (51). The neonatal lung evidently tries to adapt to enhanced oxidant stress by induction of MnSOD and possibly of ECSOD.

Carcinogenesis and the Expression of SODs in Lung Tumors

SODs can be hypothesized to play a role in carcinogenesis due to multiple effects of reactive oxygen species on cell growth and survival. Most of these studies, however, have been conducted *in vitro*. A recent study on CuZnSOD and numerous earlier studies on MnSOD have suggested that CuZnSOD and MnSOD genes are tumor suppressive (291–293). The primary findings in these experimental studies, conducted on cultured cells containing isolated overexpression of CuZnSOD or MnSOD (in most of the cases) by transfection, were suppression of neoplastic transformation, decreased proliferation, increased differentiation *in vitro*,

and—if inoculated to nude mice—decreased tumor growth (293–300). A low level of SODs in cancer cells has been postulated to be associated with specific oncogene products regulated by the redox state of the cell (159, 301), inhibition of transcription factors such as activator protein-1 and nuclear factor κ B (301, 302), and methylation of the upstream transcriptional regulatory sequence of the MnSOD gene (301, 303). Conclusions obtained from *in vitro* studies cannot be strictly extrapolated to the *in vivo* situation where several antioxidant enzymes and related detoxification mechanisms may be simultaneously induced. Highly metabolically active tumor cells also have complex interactions with numerous nonmalignant cells that regulate tumor growth and invasion (i.e., vasculature and angiogenesis) where the oxygen environment may also be highly variable.

A small immunohistochemical study on various lung malignancies (altogether 19 cases) has suggested that CuZnSOD is low in lung cancer in general (34). A recent study where lung tumor samples were assessed for CuZnSOD using several methods (Northern blotting, Western blotting, activity) reported similar levels of CuZnSOD in lung tumor homogenates and in nonmalignant lung (304). No studies on CuZnSOD have been conducted on mesothelioma tissue, but one micro array study on a malignant mesothelioma cell line showed higher expression of CuZnSOD mRNA in malignant mesothelioma cells than in nonmalignant ones (305). Thus, there are only a few studies on CuZnSOD and human lung malignancies with variable findings on CuZnSOD expression and its suggested significance.

The first small immunohistochemical study on lung cancer (19 cases) could not show any consistent increase in MnSOD (34). However, a recent much larger study showed higher total SOD activities (200%) in lung cancer than in nonmalignant control lung. Northern blotting and Western blotting analyses confirmed this being related to MnSOD (304). Thus, MnSOD is possibly elevated in lung cancer.

Several studies have investigated MnSOD in mesothelioma. These studies have demonstrated that mesothelioma contains high levels of MnSOD mRNA, protein, and activities compared with nonmalignant mesothelium or mesothelial cells, increasing threefold to 10-fold (306–309). MnSOD has been suggested to be a possible diagnostic marker of mesothelioma due to its very intense activity in mesothelioma compared with the much lower levels commonly found in adenocarcinoma metastasized to pleura (307). Thus, it is known that MnSOD is higher in malignant mesothelioma than in the nonmalignant mesothelium, but its significance for tumor promotion and drug resistance is unresolved.

A summary of the expression of SODs in human lung diseases has been given in Table 3.

TABLE 3. SUMMARY OF THE CHANGES IN EXPRESSION OF SUPEROXIDE DISMUTASES IN HUMAN LUNG DISEASES

MnSOD	
	Lowered or unchanged in asthmatic airways
	Elevated in alveolar type II epithelial cells and macrophages of interstitial pneumonias and sarcoidosis
	Low in fibroblasts and fibromyxoid lesions in usual interstitial pneumonia (idiopathic pulmonary fibrosis)
	High in the proliferating type II epithelial cells in bronchopulmonary dysplasia (chronic lung disease)
	High in mesothelioma
	Possibly elevated in lung cancer
CuZnSOD	
	Decreased in asthmatic airways
	Elevated mRNA in cultured mesothelioma cells (micro array study)
ECSOD	
	Positive in hyaline membranes and in the proliferating type II epithelial cells in respiratory distress syndrome and bronchopulmonary dysplasia (chronic lung disease)

For definitions of abbreviations *see* Table 2.

CONCLUSIONS AND FUTURE ASPECTS

A basic understanding about the expression and regulation of antioxidant enzymes in normal lung and the changes that occur in lung diseases is necessary to develop therapeutic interventions to control oxidative stress in the lung. Oxidant/antioxidant balance in the bronchial epithelium, alveolar structures, and interstitium constitute the primary defense against oxidant stress of the lung. Although the expression of SODs has been characterized relatively well in animal and human lung, the specific role of the antioxidant enzymes in the pathogenesis of most lung diseases remains unclear. For instance, individual variability of these enzymes (polymorphisms) leading to lower antioxidant activity in lung cells may be hypothesized to play a role in the development of both nonmalignant and malignant lung diseases. Several polymorphisms have in fact been characterized, but their role in the pathogenesis of various lung disorders has not yet been systematically investigated. Antioxidant enzymes exhibit cell-specific expressions in human lung. MnSOD is upregulated in inflammatory areas of some lung diseases, but whether induction offers any protection in these diseases or whether induction is a secondary reaction with variability in individuals and various cell types also remains to be investigated. ECSOD is uniquely expressed in the lung and holds potential importance in multiple human lung diseases, with special interest being directed toward interstitial lung diseases and diseases of the vasculature. The significance of ECSOD in lung diseases also waits for future investigations. More efforts should be directed not only toward understanding the significance of the variability of these enzymes in individuals and various diseases but also to their potential role in therapeutic approaches. It, however, is clear that oxidants are associated with most lung diseases and that severe ongoing oxidant stress causes downregulation of SODs. These phenomena may further enhance the progression of the lung disease. It may, therefore, be hypothesized that therapeutic antioxidants such as SOD mimetics might provide new approaches to attenuate oxidant-associated lung injury in humans. These new agents show efficacy in oxidant-induced experimental lung models, but their significance has not yet been investigated in human lung.

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