**Differential Regulation of Antioxidant Enzymes in Response to Oxidants**

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We have demonstrated the selective induction of manganese superoxide dismutase (MnSOD) or catalase mRNA after exposure of tracheobronchial epithelial cells in vitro to different oxidant stresses. Addition of H₂O₂ caused a dose-dependent increase in catalase mRNA in both exponentially growing and confluent cells. A 3-fold induction of catalase mRNA was seen at a nontoxic dose of 250 μM H₂O₂. Increase in the steady-state mRNA levels of glutathione peroxidase (GPX) and MnSOD were less striking. Expression of catalase, MnSOD, and GPX mRNA was highest in confluent cells. In contrast, constitutive expression of copper and zinc SOD (CuZnSOD) mRNA was greatest in dividing cells and was unaffected by H₂O₂ in both exponentially growing and confluent cells.

MnSOD mRNA was selectively induced in confluent epithelial cells exposed to the reactive oxygen species-generating system, xanthine/xanthine oxidase, while steady-state levels of GPX, catalase, and CuZnSOD mRNA remained unchanged. The 3-fold induction of MnSOD mRNA was dose-dependent, reaching a peak at 0.2 unit/ml xanthine oxidase. MnSOD mRNA increases were seen as early as 2 h and reached maximal induction at 24 h. Immunoreactive MnSOD protein was produced in a corresponding dose- and time-dependent manner. Induction of MnSOD gene expression was prevented by addition of actinomycin D and cycloheximide. These data indicate that epithelial cells of the respiratory tract respond to different oxidant insults by selective induction of certain antioxidant enzymes. Hence, gene expression of antioxidant enzymes does not appear to be coordinately regulated in these cell types.

Reactive oxygen species (ROS)† have been implicated in many lung diseases including those associated with exposure to asbestos, nitrogen dioxide, ozone, paraquat, hyperoxia, carbon tetrachloride, and the anticancer drugs bleomycin and adriamycin (for review see Ref. 1). Although sources of ROS and antioxidant enzymes (AOE) have been well-documented in the lung, the individual cell types involved in oxidant injury and defense are poorly understood (2–5). Phagocytic cells have been implicated in the generation of ROS during inflammation (reviewed in Ref. 6), but the functional characteristics of the cells of the lung that contribute to lung defense from oxidants are enigmatic. Conceivably, oxidant damage results when the AOE defense mechanisms of the lung are overwhelmed.

Eukaryotes have evolved several different AOE to detoxify ROS. Copper, zinc superoxide dismutase (CuZnSOD) is located primarily in the cytoplasm, whereas manganese SOD (MnSOD), a structurally distinct protein encoded by a different nuclear gene (7), is located primarily in the mitochondria. Both enzymes catalyze the reaction: O₂ + O₂ + 2H⁺ = O₂ + H₂O₂, H₂O₂ is converted to H₂O in the peroxisomes by the AOE, catalase, and in the cytoplasm by glutathione peroxidase (GPX). The distribution and regulation of AOE in cells of the lung are unknown.

Tracheal epithelial cells line the airways of the upper respiratory tract and are the first cells to encounter foreign material impinging upon the walls of the airways. Their regulation is important in understanding the defense mechanisms that occur in response to inhaled oxidants. In this study we examined the *in vitro* effect of two oxidant stresses: H₂O₂ and a xanthine/xanthine oxidase generating system producing a spectrum of ROS (O₂⁻, 'OH, H₂O₂) on the levels of steady state mRNA of MnSOD, CuZnSOD, GPX, and catalase in hamster tracheal epithelial cells (HTE; 8). The dose- and time-dependent regulation of these enzymes in response to oxidants was explored initially in both exponentially growing and confluent HTE cells. Our data indicate that the steady state message levels of these enzymes vary independently with the cell cycle. Since more dramatic increases in gene expression were seen with MnSOD in the xanthine/xanthine oxidase generating system, we used actinomycin D and cycloheximide, respectively, to explore the dependence of mRNA accumulation on transcription and protein synthesis. Western blot analysis was used to examine whether transcriptional changes in MnSOD were manifested at the protein level. Differential and unique regulation of the AOE message levels occurred in HTE cells after exposure to different oxidant stresses. Changes in CuZnSOD mRNA levels were not apparent with either oxidant.

**METHODS AND MATERIALS**

*Cell Culture Methods*—HTE cells, isolated and characterized previously (8) as a diploid, nontumorigenic cell line, were grown in Ham's F12 medium (GIBCO-Bethesda Research Laboratories) containing...
10% fetal bovine serum (Sigma, serum endotoxin level <0.125 ng/ml), 10 units/ml penicillin, and 10 μg/ml streptomycin.

**Oxidant Stresses**—Two different sources of oxidants were used at nontoxic concentrations as determined previously in HTE cells (9). H2O2 (Sigma) was diluted in phosphate-buffered saline and the concentration determined by absorbance at 240 nm (absorbance = 1.31 for 30 mM H2O2). H2O2 was added directly to fresh serum-containing medium of confluent cell culture dishes. 

ROS were generated continuously by the enzyme-substrate mixture xanthine (Sigma, grade V) and xanthine oxidase (Calbiochem). Xanthine oxidase, under aerobic conditions, catalyzes the oxidation of xanthine to uric acid with reduction of O2 to O2- (10). In a neutral aqueous environment, O2- reacts with dissolved O2 to yield HO2. Furthermore, the iron catalyzed Haber-Weiss reaction or O2- driven Fenton reaction will produce ·OH from O2 and H2O2 under these conditions (11).

Prior to addition of xanthine/xanthine oxidase, confluent cultures were given fresh serum and 20 μM xanthine containing medium. Xanthine oxidase was added at the indicated concentrations (0.005-0.4 unit/ml) and times (2-48 h). Controls consisted of cells grown in serum and 50 μM xanthine containing medium.

Preliminary experiments showed that the induction of MnSOD mRNA using the xanthine/xanthine oxidase system fluctuated depending on the sources of the enzyme and fetal bovine serum. Five lots of serum and three lots of xanthine oxidase from different suppliers were assayed for comparative induction of MnSOD mRNA (data not shown). Since production of superoxide by various lots of xanthine oxidase varied, one lot of xanthine oxidase and serum were used for all experiments.

**RNA Isolation and Northern Blots**—Total RNA was isolated from cells using the procedure of Chomczynski and Sacchi (12). RNA was resolved by electrophoresis in a 1% agarose, 2.25 M formaldehyde gel in a running buffer containing 20 mM MOPS, pH 7.4, and 1 mM EDTA. RNA was transferred to nitrocellulose (Schleicher and Schuell, Keene, NH) according to Maniatis et al. (13). Alternatively, total RNA was applied directly to nitrocellulose using a slot blot apparatus (Schleicher and Schuell; 13). Purified cDNAs were labeled with [α-32P]dATP (3,000 Ci/mmole, DuPont-New England Nuclear) by random hexamer priming using a Prime-a-Gene kit (Promega, Madison, WI). Blots were prehybridized 2-12 h in 50% formamide, 0.1% SDS at 42 °C. Blots were hybridized with cDNAs, labeled to a specific activity of 108 cpn/μg, in hybridization fluid at 42 °C overnight. Hybridized blots were washed in 0.5 M sodium chloride, 0.05 M sodium citrate, pH 7.0, 0.1% SDS at 42 °C and monitored with a Geiger counter. More stringent washing conditions were used as needed. Autoradiographs were made by exposing blots to x-ray film (Kodak XAR-5), at -70 °C with an intensifying screen. Radioactivity on blots was quantified directly by detection in a Betascope Blot Analyzer, Model 603, version 2.0, and by Geiger counter. Autoradiography of the autoradiographs using a Microscan 1000, Technology Resources Inc., Nashville, TN. Samples analyzed using the Betascope were expressed as counts accumulated. These were varied for varying amounts of time, typically 6-14 h, depending upon the length and specific activity of the probe, which is similar to the exposure time frame for the autoradiographs.

Dr. Guy Mullenbach (Chiron Corporation, Emeryville, CA) provided the murine GPX cDNA containing plasmids, pmpGPx-9A, and pmpGPx-5A (14). From Dr. Ye-Shih Ho (Duke University, Medical Center, Durham, NC) we obtained a 0.65-kb rat CuZnSOD cDNA containing plasmid, pUC19-BSC (15), and a 1.4-kb rat MnSOD cDNA containing plasmid, pSP65-RMS (16). The murine catalase probe, pmCAT-34 (17) was given to us by Dr. Jacqueline Shaffer (State of New York Department of Health, Albany, NY).

**Inhibition of Protein and RNA Synthesis**—HTE cells were exposed to nontoxic concentrations of inhibitors for 24 h. Fifty μM cycloheximide (Sigma) was used to inhibit protein synthesis and 4 μM actinomycin D (Sigma) was used to inhibit RNA synthesis (18). ROS exposed cells received 0.2 unit/ml xanthine oxidase.

**Western Blot Analysis**—Confluent epithelial cells were exposed to 50 μM xanthine (control cultures) and 0.2 unit/ml xanthine oxidase in serum containing medium for 24 or 48 h. After aspiration of medium, cell layers were washed 2× with calcium and magnesium free phosphate buffered saline (GIBCO-BRL, CMF-PBS). Cells were scraped off the dishes with the aid of a rubber policeman in CMF-PBS, at 4 °C, and washed 2× more in CMF-PBS. Cells were frozen in equal volumes of deionized water and lyophilized. Prior to electrophoresis, samples were dissolved in a sample buffer (62.5 mM TrisCl, pH 6.8, 2% SDS, 10% glycerol, and 5% β-mercaptoethanol) and boiled 3 min before loading on the gel. Aliquots saved prior to lyophilization were used for protein determination (19) using bovine serum albumin, Fraction V (Sigma), as a standard. Samples including prelabeled molecular weight standards (GIBCO-BRL) were electroblotted in 15% SDS on a 0.45 μm pore size in Nylon II Cell apparatus (Bio-Rad) and transferred to nitrocellulose (Schleicher and Schuell) as described previously (20). The nitrocellulose blot was stained with anti-MnSOD antibody diluted in 0.05% Tween-20 (Sigma) in CMF-PBS. Secondary antibody, biotinylated goat anti-rabbit IgG, peroxidase-conjugated avidin, and 4-chloro-1-naphthol, were supplied in kit form (Vector Laboratories Inc., Burlingame, CA) and used according to the recommendation of the supplier.

Anti-human kidney MnSOD antibody (21) was generously provided by Dr. Larry W. Oberley (University of Iowa, Iowa City, IA).

**RESULTS**

Effect of H2O2 on AOE mRNA Levels—The addition of H2O2 to HTE cells caused a dose-dependent increase in the levels of steady state message for catalase (Fig. 1). This response was observed in exponentially growing cells as well as in cells which had reached confluence prior to exposure to H2O2. The greatest induction was observed with 100 and 250 μM H2O2, which resulted in increases of 2.5- and 5-fold, respectively, in the message levels of catalase mRNA. The message levels of GPX, which also catalyzes the reduction of H2O2, were less responsive to H2O2. Cells growing exponentially did not demonstrate any change in the levels of GPX mRNA in response to H2O2, but at concentrations of 100 and 250 μM H2O2, the levels of GPX mRNA increased by as much as 50% in confluent cells. Exposure of HTE cells to H2O2 (100 μM) during log phase-growth had relatively little effect on the steady state levels of MnSOD mRNA (Fig. 1). However, 250 μM H2O2 caused a 40% increase in the level of MnSOD mRNA in confluent HTE cells. The CuZnSOD message level was not modified by 100-250 μM H2O2 whether cells were growing exponentially or had reached confluence prior to exposure.

**Expression of AOE mRNA Levels during the Cell Cycle**—Comparing unexposed HTE cultures at log phase and confluence revealed unique and differential expression of AOE mRNA levels (Fig. 1). GPX mRNA levels were comparable in rapidly growing and confluent epithelial cells. By contrast, the levels of mRNA for catalase increased 4-5-fold when actively growing cells reached confluence. Similarly, MnSOD mRNA increased 3-fold as HTE cells advanced from log phase to confluence. In contrast, levels of CuZnSOD mRNA displayed an opposite pattern. Upon reaching confluence, the steady-state level of message for this AOE decreased to approximately half the level observed during exponential growth.

The cell cycle variation in steady state message levels of AOE did not appear to influence patterns of regulation when HTE cells were challenged by an oxidant stress such as exposure to H2O2. For example, catalase mRNA was induced by H2O2 in both exponentially growing and confluent cells. Based on these results and the higher overall levels of AOE mRNA in confluent cells, the following experiments were conducted with confluent cell cultures, a system which mimics the contiguous cell layer of the tracheal epithelium in vivo.

**Effect of Xanthine/Xanthine Oxidase on AOE mRNA Levels**—The production of ROS during the oxidation of xanthine by xanthine oxidase was exploited as a source for the continuous generation of these species, including O2·- (10). The steady state levels of MnSOD mRNA demonstrated a dose-dependent increase after exposure of HTE cells to xanthine/xanthine oxidase (Fig. 2). Peak induction of MnSOD mRNA was seen with addition of 0.2 unit/ml xanthine oxidase where
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FIG. 1. Northern analysis of mRNA from HTE cells exposed to H$_2$O$_2$. Cells were exposed for 18 h to varying concentrations of H$_2$O$_2$ during log phase of growth or at confluence. Fifteen µg of total RNA per lane were electrophoresed, transferred to nitrocellulose, and hybridized as described under "Materials and Methods." A, autoradiographs of Northern blots hybridized sequentially with cDNAs to catalase, GPX, MnSOD, and CuZnSOD. Duplicate samples represent separate cell culture dishes. B, quantitative data from Betascope analysis or densitometric scanning (catalase). The bars represent the average of the duplicate samples seen in A.

levels increased 2–3-fold after 18 h in comparison to unexposed cells.

Examination of the other AOE revealed that this induction was unique to MnSOD mRNA. The message levels of the other three AOE did not increase, but rather showed a slight downward trend. These data indicate that this oxidant stress is specific in its selective induction of MnSOD mRNA. Because MnSOD has been implicated in inflammatory disease and is induced by endotoxin and cytokines such as TNF and IL-1 (18, 22, 23), induction of MnSOD mRNA in HTE cells was studied further.

Time Course of MnSOD mRNA Induction—A time course was performed using the most effective concentration of 0.2 units/ml xanthine oxidase. HTE cells demonstrated induction of MnSOD mRNA which peaked at 24 h and returned to control levels by 48 h (Fig. 3). With the exception of a transient spike in catalase mRNA levels at 2 h, the other three AOE message levels remained constant after exposure to xanthine/xanthine oxidase.

Effects of Actinomycin D and Cycloheximide on MnSOD mRNA—In order to evaluate the contribution of transcription to the induction of MnSOD mRNA, we used the RNA synthesis inhibitor, actinomycin D. Addition of actinomycin D to control HTE cells (without oxidant exposure) resulted in a
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Fig. 3. Time course of induction of MnSOD mRNA in HTE cells. Confluent cells were exposed to 50 μM xanthine plus 0.2 unit/ml xanthine oxidase for 2, 4, 8, 24, and 48 h. One μg of total RNA was applied directly to nitrocellulose using a slot blot apparatus as described under "Materials and Methods." Quantitative data were collected from Betascope analysis of slot blots hybridized with cDNAs to MnSOD, CuZnSOD, GPX, or catalase. Autoradiograph images of representative slots are beneath each corresponding graph. Control = 50 μM xanthine exposed cells; X/XO = xanthine/xanthine oxidase exposed cells.

Fig. 4. Effect of actinomycin D and cycloheximide on the induction of MnSOD mRNA in HTE cells. Confluent cells were exposed to 50 μM xanthine plus 0.2 unit/ml xanthine oxidase with the further addition in the indicated samples of 50 μM cycloheximide or 4 μg actinomycin D, for 24 h. One μg of total RNA was applied to nitrocellulose using a slot blot apparatus. 4.5-fold increase in MnSOD mRNA levels (Fig. 4). This was an unexpected finding and might suggest that the turnover of MnSOD mRNA requires mRNA synthesis. Exposure of cells to actinomycin D concurrently with xanthine/xanthine oxidase resulted in a 20% decrease in MnSOD mRNA levels when compared with HTE cell exposed to actinomycin D alone. This suggests that the induction of MnSOD mRNA observed with xanthine/xanthine oxidase requires active transcription of mRNA.

The involvement of protein synthesis was examined by addition of cycloheximide to HTE cells (Fig. 4). Addition of cycloheximide resulted in a 10-fold increase in message levels of MnSOD. However, addition of both cycloheximide and xanthine/xanthine oxidase to HTE cells did not cause a further increase in the level of MnSOD mRNA when compared with cells exposed to cycloheximide alone. Xanthine/xanthine oxidase and cycloheximide exposed cells contained 20% of the MnSOD message compared with control HTE cells (cycloheximide alone). These observations suggest involvement of protein synthesis in the induction of MnSOD message.

HTE cells exposed to ROS generated by the xanthine/xanthine oxidase system demonstrated an induction of MnSOD immunoreactive protein (Fig. 5). Amounts of MnSOD protein increased in a dose- and time-dependent manner. The 2–3-fold increase in immunoreactive MnSOD correlated well with the 2–3-fold increase in MnSOD mRNA (Figs. 2 and 3). This implies that induction of MnSOD mRNA results in a corresponding increase in MnSOD protein.

DISCUSSION

Epithelial cells of the respiratory tract function in an oxygen-rich environment and must deal continuously with air-
borne foreign material. These cells must maintain a balance between the normal encounter of oxidants (e.g., through release by phagocytic cells) and antioxidant defense mechanisms (9). Removal of toxic oxygen metabolites is the putative function of AOE such as CuZnSOD, MnSOD, GPX, and catalase. The regulation of these enzymes is critical to our understanding of inflammation and oxidant-associated lung disease.

In our studies, HTE cells responded to different oxidants by differential regulation of AOE. In the presence of H$_2$O$_2$, these cells showed a 3-fold increase in catalase mRNA (Fig. 1), in both exponentially growing and confluent cells.

We were also interested in the effects of a spectrum of ROS on AOE regulation, using a xanthine/xanthine oxidase generating system. Fridovich (10) first characterized the univalent reduction of O$_2$ by xanthine oxidase, demonstrating simultaneous one- and two-electron reactions under physiological conditions. Approximately 70% of the O$_2$ consumed was observed to be reduced to H$_2$O$_2$ and 30% of the O$_2$ reduced univalently to O$_2^-$. The xanthine/xanthine oxidase system has been re-examined more recently by Kuppusamy and Zweier (24). Using electron paramagnetic resonance spectroscopy, these authors demonstrated the generation of $OH$ through the non-iron mediated univalent reduction of H$_2$O$_2$. Thus, the xanthine/xanthine oxidase mixture has been employed in studies here for the continuous generation of short lived oxygen radicals, O$_2^-$ and 'OH, along with the oxygen metabolite, H$_2$O$_2$.

The response to oxidants by HTE cells is discriminating since the challenge presented by the xanthine/xanthine oxidase generating system results in a completely different pattern of AOE mRNA levels than that of H$_2$O$_2$. After exposure to xanthine/xanthine oxidase, MnSOD mRNA is selectively increased in a dose- and time-dependent manner (Figs. 2 and 3). In contrast, the other three AOE examined remained unchanged or decreased slightly when their steady state mRNA levels were measured.

Numerous mechanisms could account for these differences. The involvement of cytokines in the regulation of MnSOD has been documented in recent years. For example, human melanoma A375 cells, skin fibroblasts, and peripheral blood monocytes increase their synthesis of MnSOD when exposed to interleukin-1 (IL-1; 25). MnSOD mRNA is selectively induced in a variety of cell types by tumor necrosis factor $\alpha$ and $\beta$ (TNF) as well as IL-1$\alpha$ and $\beta$ (22). In these studies, catalase, CuZnSOD, and GPX mRNAs were unchanged by TNF-$\alpha$.

The role of oxidant stress in the regulation of message levels of MnSOD has recently been examined by Visner et al. (18), who demonstrated the selective induction of MnSOD mRNA, but not CuZnSOD mRNA, in rat pulmonary epithelial cells exposed to lipopolysaccharide (endotoxin), IL-1, or TNF. A 24-h exposure of epithelial or fibroblast cells to 95% O$_2$ did not alter the mRNA levels of either MnSOD or CuZnSOD. These observations are in agreement with Eqbal et al. (26), who examined hyperoxic exposure of rats and found an increase in CuZnSOD mRNA only occurred in animals pre-exposed to endotoxin for 48 h prior to exposure to 95% O$_2$.

In our work and in studies by others (18), actinomycin D was employed as a general RNA synthesis inhibitor in order to dissect the transcriptional dependence of the induction of MnSOD mRNA (Fig. 4). Interestingly, actinomycin D caused an increase in MnSOD mRNA in control HTE cell cultures. This suggests RNA synthesis is required to regulate the turnover of the message for MnSOD. Perhaps a regulatory RNA molecule or nuclease exists and inhibiting its synthesis stabilizes the MnSOD message or inhibits MnSOD mRNA turnover. No further induction of MnSOD mRNA by the xanthine/xanthine oxidase generating system was observed in actinomycin D-exposed cells. This observation suggests that transcription is required for the increase of MnSOD mRNA synthesis in this system.

Inhibition of protein synthesis by cycloheximide caused an even greater increase in MnSOD mRNA (Fig. 4). Comparable induction of MnSOD mRNA by cycloheximide was also seen when rat pulmonary epithelial-like cells were exposed to endotoxin (18). This phenomenon suggests a protein component is involved in the turnover of this message. There are several possibilities to consider. A repressor protein with a short half-life may actively regulate the concentration of MnSOD mRNA. Inhibition of its synthesis would account for the accumulation of MnSOD mRNA observed. Alternatively, proteins may be involved in the degradation of MnSOD mRNA. Thus, inhibition of protein synthesis would allow for the accumulation of this message. No further increase in MnSOD mRNA was observed when cycloheximide and xanthine/xanthine oxidase were added simultaneously to HTE cells, indicating requirement of protein synthesis for induction of MnSOD mRNA. A combination of agents resulted in only a 2-fold induction of message which is less than that observed with cycloheximide alone (Fig. 4). Thus, it is possible that the cadre of ROS released by the xanthine/xanthine oxidase mixture results in an attenuation of cycloheximide's inhibition of protein synthesis.

The induction of MnSOD mRNA observed with xanthine/xanthine oxidase was coupled to a functional increase in MnSOD protein which was dose and time-dependent (Fig. 5). The pattern of induction of the immunoreactive protein paralleled increases in steady-state levels of mRNA. However, increases in immunoreactive MnSOD persisted after 48-h exposure to ROS, whereas the MnSOD mRNA steady state levels were returning to control levels by 48 h. Allowing for lag time following transcriptional increases in MnSOD gene expression, the MnSOD data correlate well with the mRNA data (compare Figs. 2, 3, and 5).

The work presented here demonstrates the selective induction of mRNA for specific AOE with different oxidants. Exposure of epithelial cells to H$_2$O$_2$ caused a significant induction of catalase mRNA, and at higher concentrations, a detectable increase in GPX mRNA. Alternatively, ROS generated by xanthine/xanthine oxidase were associated with the specific induction of MnSOD mRNA. Although H$_2$O$_2$ is generated from this system, H$_2$O$_2$ alone accounts for only a 40% increase in MnSOD mRNA over control values (see Fig. 1), implicating the other ROS generated in the 3-fold increase in MnSOD mRNA observed (Figs. 2 and 3). In contrast to studies by others using cytokines, endotoxin, or hyperoxia as an oxidant stress (18, 22, 23), we used direct addition of oxidants to evaluate the effect on AOE gene regulation. Our data indicate that the CuZnSOD isoform is not inducible by ROS nor coordinately regulated with MnSOD in epithelial cells of the respiratory tract.

Recently the rat gene for MnSOD has been isolated, and some of its molecular structure described (7). No mention was made regarding the presence or absence of previously characterized elements responsive to oxidative stress in prokaryotes or the antioxidant response element described recently in eukaryotes (27, 28). The oxyR gene encodes a protein regulator controlling nine proteins with expression inducible in bacteria following exposure to H$_2$O$_2$ (29). This oxyR protein acts as a transcriptional activator only in its oxidized form (29). In addition, the soxR regulon has been described in
bacteria, as responsible for the positive control of nine proteins induced by O$_2^-$ and distinct from those induced by H$_2$O$_2$ (30). With the MnSOD sequence defined, the regulatory elements responsible for induction of MnSOD gene expression by various oxidant stresses can now be identified, using the prokaryotic systems by way of example.

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REFERENCES
1. Mossman, B. T., and Marsh, J. P. (1989) Environ. Health Perspect. 81, 91–94
2. Fantone, J. C., and Ward, P. A. (1982) Am. J. Pathol. 107, 397–418
3. Freeman, B. A., and Crapo, J. D. (1982) Lab. Invest. 47, 5–18
4. Heffner, J. E., and Repine, J. E. (1989) Am. Rev. Respir. Dis. 140, 531–554
5. Sibelle, Y., and Reynolds, H. Y. (1990) Am. Rev. Respir. Dis. 141, 471–501
6. BaggioIini, M., and Wymann, M. P. (1990) Trends Biochem. Sci. 15, 69–72
7. Ho, Y.-S., Howard, A. J., and Crapo, J. D. (1991) Am. J. Respir. Cell Mol. Biol. 4, 278–286
8. Mossman, B. T., Ezerman, E. B., Adler, K. B., and Craighead, J. E. (1986) Cancer Res. 46, 4409–4409
9. Marsh, J. P., and Mossman, B. T. (1991) Cancer Res. 51, 167–173
10. Fridovich, I. (1970) J. Biol. Chem. 245, 4053–4057
11. Grisham, M. B., and McComb, J. M. (1986) Physiology of Oxygen Radicals (Taylor, A. E., Matalon, S., and Ward, P. A., eds) pp. 1–18, Waverly Press, Baltimore
12. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
13. Maniatis, T., Frisch, E. F., and Sambrook, J. (1980) Molecular Cloning: A Laboratory Manual. pp. 201, 383–386, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
14. Mullenbach, G. T., Tabrizi, A., Irvine, B. D., Bell, G. I., Trainer, J. A., and Hallewell, R. A. (1988) Oxy-radicals in Molecular Biology and Pathology (Cerutti, P. A., Fridovich, I., and McCord, J. M., eds) pp. 313–326, Alan R. Liss, Inc., New York
15. Ho, Y.-S., and Crapo, J. D. (1987) Nucleic Acids Res. 15, 6746
16. Ho, Y.-S., and Crapo, J. D. (1987) Nucleic Acids Res. 15, 10070
17. Shaffer, J. B., Sutton, R. B., and Bewley, G. C. (1987) J. Biol. Chem. 262, 12908–12911
18. Visner, G. A., Douall, W. C., Wilson, J. M., Burr, I. A., and Ando, K. (1989) Proc. Natl. Acad. Sci. U. S. A. 87, 6181–6185