Regulation of Manganese Superoxide Dismutase and Other Antioxidant Genes in Normal and Leukemic Hematopoietic Cells and Their Relationship to Cytotoxicity by Tumor Necrosis Factor

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Myeloid cells are a major source of superoxide and other oxygen metabolites. As a protective mechanism, cells express antioxidant enzymes including manganese superoxide dismutase (Mn-SOD), copper-zinc SOD (Cu/Zn-SOD), and glutathione peroxidase (GSX-PX). Even though hematopoietic cells are a major source of oxidants, little is known of their expression of antioxidants. We found that seven myeloid leukemic cell lines blocked at different stages of differentiation constitutively expressed Mn-SOD, Cu/Zn-SOD, and GSX-PX RNAs. Level of Mn-SOD activities paralleled levels of Mn-SOD RNA. Terminal differentiation of native HL-60 cells to either granulocytes or macrophages did not alter levels of Mn-SOD RNA but markedly decreased cell division. Myeloid leukemic lines sensitive to cytotoxic effects of tumor necrosis factor (TNF) as well as normal peripheral blood lymphocytes and monocytes, dramatically increased their levels of Mn-SOD RNA in the presence of TNF. In contrast, Cu/Zn-SOD and GSX-PX RNA levels did not increase in these same cells. TNF-resistant leukemic lines had higher constitutive levels of Mn-SOD RNA and activity; and these levels did not change in the presence of TNF. Antisense but not random oligonucleotides to Mn-SOD markedly increased the sensitivity to the inhibitory effects of TNF for both the native HL-60 (TNF-sensitive) and K562 (TNF-resistant) cell lines. Further studies showed that the antisense oligonucleotides entered the cells and resulted in decreased levels of Mn-SOD RNA. The data suggest that Mn-SOD may provide protection against cytotoxicity of TNF in hematopoietic cells.

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MATERIALS AND METHODS

Cells. Leukemic cell lines used in this study were as follows: KG-1 (myeloblasts), native HL-60 (promyelocytes), ML-3 (early...
myelomonoblasts), THP-1 and U937 (monoblasts), and K562 and HEL (early myeloid/erythroid blast cells). W138 (normal human embryonic lung fibroblasts) and Lu-CSF-1 cells (human adenocarcinoma of lung) (obtained from American Type Tissue Culture Collection [ATCC], Bethesda, MD) were also used. The TNF-resistant native HL-60 cells were developed in our laboratory. Briefly, native HL-60 cells were plated at 1 × 10^6 cells per 100-mm dish in alpha medium with 10% fetal bovine serum (FBS), and TNF was added to the cultures at increasing concentrations from 20, 50, 100, 300, to 1,000 U/mL each for 3-week intervals. During culture with TNF, medium containing freshly prepared TNF was added every 3 days. After exposure to 1,000 U/mL TNF, the TNF-resistant native HL-60 cells were isolated by growing the cells in 1,000 U/mL TNF in soft gel culture, and an individual clone (HL-60R) was expanded in liquid culture. These cells expressed normal levels of TNF receptors (data not shown). 16

Mononuclear blood cells were isolated by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density separation from heparinized venous blood obtained from healthy donors after receiving informed consent. These cells were washed twice with phosphate-buffered saline (PBS). Monocytes were removed by their ability to adhere by culturing cells on plastic dishes for 3 hours. The nonadherent, mononuclear fraction contained greater than 90% lymphocytes, as determined by light microscopy of Wright-Giemsa-stained slide preparation. The adherent cells were greater than 95% monocytes as determined by morphologic examination using light microscopy of Wright-Giemsa-stained slide and positive staining with α-naphthyl butyrate esterase. Cells were cultured in alpha medium (Flow Laboratories, Inc, Rockville, MD) supplemented with 10% FBS (Irvine Scientific, Santa Ana, CA) in a humidified atmosphere with 5% CO2.

Chemicals. Recombinant human TNF-α (5.6 × 10^7 U/mg protein) was supplied by Genentech (San Francisco, CA). Specific activity was assayed by examining cytolytic action on actinomyacin D–treated L929 fibroblasts. 17

All trans-retinoic acid (RA), dimethyl sulfoxide (DMSO), and 12-O-tetradecanoylphorbol 13 acetate (TPA) were purchased from Sigma Chemical Co, St Louis, MO, dissolved in either ethanol or acetone and stored at −20°C. The 1,25-dihydroxyvitamin D3 ([1,25(OH)2D3]), provided by Dr M. Uskokovic (Hoffmann-LaRoche, Nutley, NJ) was dissolved in ethanol and stored at −20°C.

Northern blot analysis. Total cellular RNA was extracted from hematopoietic cell lines by hot phenol extraction. 18 Twenty micrograms of RNA per sample was electrophoresed on formaldehyde-agarose gels (Bethesda Research Laboratories, Gaithersberg, MD), and transferred to nylon membrane (ICN Biomedicals Inc, Irvine, CA). Hybridization with the labeled probes was for 16 to 24 hours at 42°C in 50% formamide, 2× SSC (1× SSC = 150 mmol/L NaCl, 15 mmol/L sodium citrate, pH 7.0), 5× Denhardt’s, 0.1% sodium dodecyl sulfate (SDS); 10% dextran sulfate (Sigma Co). Filters were washed to a stringency of 0.1× SSC at 65°C and exposed to Kodak XAR film (Eastman Kodak, Rochester, NY). Autoradiograms were exposed for 1 to 7 days. Modulation in levels of SOD RNA was quantified by initial standardization to the amount of β-actin-specific transcripts. The relative density of β-actin–specific and SOD–specific transcripts in the different lanes was first determined by laser densitometry using multiple exposure of blots and the ratio of SOD/β-actin in the control lane was assigned to be the basal level. The fold-stimulation in the experimental lanes was calculated by the reciprocal of the ratio of the base level.

A human Mn-SOD cDNA probe (EcoRI-EcoRI; 1.0 kb) was provided by Dr K. Matsuishi (National Cancer Institute, Bethesda, MD) and human GSX-PX cDNA probe (Pst I-EcoRI; 0.6 kb) was provided by Dr M. Mirault (Quebec, Canada). A human Cu/Zn-SOD cDNA probe (BamHI-BamHI; 0.8 kb) was obtained from ATCC; and β-actin probe was EcoRI-BamHI (0.7 kb) fragment from plasmid pHf A-3'ut.I. These probes were 32P-labeled by random priming.

Cellular incorporation of radioactive thymidine. The native HL-60 cells and peripheral blood lymphocytes were incubated with 1 μCi3H-thymidine for 4 hours at 37°C; cells were washed twice in PBS, precipitated in 5% trichloroacetic acid (TCA) (30 mmol/L Na2HPO4) at 4°C for 1 hour, filtered onto glass microfilter membrane (Whatman, Hillboro, OR), washed in 3% TCA (30 mmol/L Na2HPO4) and heated at 80°C for 1 hour. Samples were assayed by liquid scintillation counter.

Mn-SOD activity. The Mn-SOD activity was assayed according to the method reported by Oberley and Spitz. 21 The ability of xanthine–xanthine oxidase generated O2 to reduce nitroblue tetrazolium (NBT) at a constant rate was used as a competitive Mn-SOD assay. The rate of NBT reduction was monitored spectrophotometrically at 560 nm. One unit of SOD was defined as the number of cells (per 10^6 cells) required to inhibit by 50%, the maximal NBT reduction. To inhibit the Cu/Zn-SOD activity, we used 5 mmol/L sodium cyanide (Sigma Co) and determined the activity of Mn-SOD.

Ager cloning assay using antisense oligonucleotides to Mn-SOD. Antisense and random oligonucleotides (oligos) to the human Mn-SOD gene were purchased from Genosys Biotechnologies, Inc (Woodlands, TX); antisense oligonucleotides (which included the transcriptional start site) were 5'-TGCGCGGCCGCTCAACTGCTGCTCTG-3'; the random oligonucleotides (control) were: 5'-GCGCGATCTTGCAGAAACA-3'. The K562 and native HL-60 cells (1 × 10^6/mL) were incubated with either antisense or random oligonucleotides (30 μmol/L) in Iscove’s modified Dulbecco’s medium (Sigma Chemical Co) without serum at 37°C for 36 hours. After the incubation, additional oligonucleotides (30 μmol/L), alpha medium and FBS (1% vol/vol for K562 cells, 3% vol/vol for native HL-60 cells) were added to cells (1 × 10^6/mL) and placed in 3% agar (Di-eco Laboratories, Inc). Native HL-60 cells were incubated with or without TNF. Cultures were incubated with 5% CO2 at 37°C and colonies were counted after 7 days. The data were compared by Student’s t-test.

Cellular uptake and stability of nucleotides. To examine oligonucleotide uptake and stability by native HL-60 cells, 5′-end-labeled antisense oligonucleotides to Mn-SOD gene were incubated for 36 hours at 37°C in PBS alone and in the presence of native HL-60 cells in alpha medium with 3% heat-inactivated FBS. The oligonucleotides were end-labeled with T4 polynucleotide kinase (Bethesda Research Laboratories, Bethesda, MD) and α-[32P]-adenosine triphosphate (ATP) to a specific activity of 1 × 10^6 cpm/μg native. Native HL-60 cells (5 × 10^6) in 100 μL alpha medium with heat-inactivated FBS were exposed to 10 μmol/L of 5′-end-labeled oligonucleotides for 36 hours at 37°C. The cells were washed twice and supernatant saved (extracellular fraction). Nonidet-P40 (Np-40) (10 mmol/L Tris.HCl, pH 8.0, 140 mmol/L NaCl, 15 mmol/L MgCl2, 0.5% NP-40 [Sigma]) was then added, and nuclei were separated by centrifugation. This phase represented the cytoplasmic fraction and was extracted twice with phenol/ chloroform, and precipitated overnight in 0.3 mol/L Na acetate (pH 5.2) and 2 vol absolute ethanol. The precipitate was then electrophoresed in 7 mol/L urea/20% polyacrylamide gel and autoradiographed.

Semi-quantitative analysis of Mn-SOD RNA by reverse transcriptase polymerase chain reaction (RT-PCR). Native HL-60 cells were incubated either with or without antisense oligonucleotides
for 36 hours, according to the same procedure used for measuring colonies. Total RNA was extracted and the expression of Mn-SOD RNA was detected by RT-PCR method. The RT reaction was performed using 1 μg of total RNA, 100 pmol of random hexamer (Boehringer-Mannheim, Indianapolis, IN), 16 U RNase inhibitor (Promega, Madison, WI), 200 U MMLV-reverse transcriptase (Bethesda Research Laboratories) and deoxynucleotides (dNTPs, final concentration 0.5 mmol/L each) in a total volume of 20 μL and under conditions recommended by suppliers. After cDNA synthesis, 35 cycles of PCR (denatured at 95°C for 1 minute, primer annealed at 60°C for 1 minute, extended at 72°C for 2 minutes) were performed with automated DNA Thermal Cycler (Perkin-Elmer/Cetus, Norwalk, CT) using sets of primers specific for Mn-SOD and β-actin in the following reaction conditions: 10 mmol/L Tris.HCl (pH 8.3), 50 mmol/L KCl, 2.5 mmol/L MgCl₂, 200 μmol/L each dNTP, 0.5 U of Taq DNA polymerase (Perkin-Elmer/Cetus) and 1 μmol/L of each primer in a total volume of 50 μL. The sequence of each primer was as follows: Mn-SOD (sense), 5'-ATGGTAGCCGAGGTGTTTCG-3'; Mn-SOD (antisense), 5'-CTGAGAGCTGCTGGGCTGT-3'. β-actin (sense), 5'-ATGGATGATGATATCGCC GCG-3'; β-actin (antisense), 5'-AAAGAACACGGCTAAGTGTGC-3'. The sequence of each primer was as follows: Mn-SOD (sense), 5'-ATGGTAGCCGAGGTGTTTCG-3'; Mn-SOD (antisense), 5'-CTGAGAGCTGCTGGGCTGT-3'. β-actin (sense), 5'-ATGGATGATGATATCGCC GCG-3'; β-actin (antisense), 5'-AAAGAACACGGCTAAGTGTGC-3'. 854 bp, PCR products (20 μL) were electrophoresed on 2% NuSieve/1% SeaKem agarose gels and visualized by staining with ethidium bromide. The specificity of PCR amplification was also examined by Southern blotting the amplified cDNA with hybridization to nonradioactive probe (ECL 3'-oligo labelling and detection system: Amersham, Arlington Heights, IL).

RESULTS

Expression of RNA for Mn-SOD and other antioxidant enzymes in human myeloid leukemia cell lines. We examined Mn-SOD, Cu/Zn-SOD and GSX-PX RNA accumulation in cells from human myeloid leukemia cell lines blocked at different stages of differentiation (early and late myeloblasts [KG-1, native HL-60]; monoblasts [U937, THP-1]; erythroblasts [K562, HEL]) (Fig 1). Constitutive expression of Mn-SOD RNA was detected in each myeloid cell line. Transcripts were 4.0 and 1.0 kb, and no variant sizes were observed in any cell line. Hybridization of the same blot with a 32P-labeled β-actin DNA probe showed that comparable amounts of RNA were present in each lane (Fig 1). The THP-1, K562, and HEL cells had the greatest accumulation of Mn-SOD RNA. Expression of Cu/Zn-SOD and GSX-PX RNA was also detectable in each myeloid line. Four lines (KG-1, ML-3, THP-1, and HEL) expressed the greatest levels of Cu/Zn-SOD RNA. The ML-3 and THP-1 lines expressed slightly less GSX-PX RNA than the other five lines. Expression of Mn-SOD activity in human leukemic cell lines. The Mn-SOD activity of cells of each of the myeloid lines in general paralleled their Mn-SOD RNA levels (Table 1). The three lines (THP-1, K562, and HEL) with the highest level of expression of Mn-SOD RNA had the highest content of Mn-SOD (22-26 U/10⁶ cells). No clear correlation between cell type and quantity of Mn-SOD was noted, except that two of three of the high-expressor lines were erythroid in phenotype. As summarized in Table 1 from prior studies by us, the three lines (THP-1, K562, and HEL) with the highest levels of SOD, were resistant to the cytotoxic/cytostatic effects of TNF. Also, all the cell lines expressed a comparable number of high-affinity TNF receptors.

Expression of Mn-SOD and Cu/Zn-SOD RNAs and 3H-thymidine incorporation during differentiation of native HL-60 cells. The native HL-60 cells can terminally differentiate along the granulocytic pathway; after 4 days exposure to either DMSO (1.25%) or RA (10⁻⁸ mol/L), approximately 70% to 80% of the native HL-60 cells matured towards granulocytic cells, as shown by morphology and ability to reduce NBT (data not shown). Also, native HL-60 cells can differentiate along the macrophage pathway; exposure to either 1,25(OH)₂D₃ (10⁻² mol/L) or TPA (10⁻⁸ mol/L) for 4 days induced approximately 85% to 95% of native HL-60 cells to differentiate toward morphologic and functional macrophagelike cells as measured by positive staining with α-naphthyl butyrate esterase (data not shown). Native HL-60 cells and those differentiated toward either granulocytes or macrophages had comparable levels of Mn-SOD RNA, (Fig 2A). The granulocytic and macrophagelike native HL-60 cells had 80% to 95% lower levels of 3H-thymidine incorporation as compared with the wild-type.

Fig 1. Expression of Mn-SOD, Cu/Zn-SOD and GSX-PX RNA in human myeloid leukemia cell lines: KG-1 (myeloblasts), native HL-60 (late myeloblasts), ML-3 (myelomonoblasts), U937, and THP-1 (monoblasts), K562 and HEL (early myeloid/erythroid blast cells). Total RNA was extracted and analyzed by Northern blot technique and hybridized with 32P-labeled Mn-SOD, Cu/Zn-SOD, and GSX-PX cDNAs, as described in Materials and Methods. Hybridization with a 32P-labeled β-actin probe was performed as a control for the amount of RNA in each lane.
Table 1. Mn-SOD Content of Various Myeloid Lines

<table>
<thead>
<tr>
<th>Myeloid Cell Lines</th>
<th>Mn-SOD Activity (U/10^6 cells)</th>
<th>Mn-SOD mRNA*</th>
<th>TNF Receptors</th>
<th>Clonal Inhibition of Cells by TNF (ED_{50}, pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KG-1 (myeloblast)</td>
<td>11.5 ± 0.2</td>
<td>+</td>
<td>676</td>
<td>31</td>
</tr>
<tr>
<td>HL-60 (late myeloblast)</td>
<td>10.3 ± 0.5</td>
<td>+</td>
<td>776</td>
<td>42</td>
</tr>
<tr>
<td>ML-3 (myelomonoblast)</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>U937 (monoblast)</td>
<td>10.2 ± 0.8</td>
<td>++</td>
<td>550</td>
<td>22</td>
</tr>
<tr>
<td>THP-1 (monoblast)</td>
<td>21.9 ± 0.4</td>
<td>+++</td>
<td>1,870</td>
<td>94</td>
</tr>
<tr>
<td>K562 (early myeloid/erythroid blast)</td>
<td>22.1 ± 0.5</td>
<td>+++</td>
<td>537</td>
<td>28</td>
</tr>
<tr>
<td>HEL (early myeloid/erythroid blast)</td>
<td>25.9 ± 1.1</td>
<td>+++</td>
<td>517</td>
<td>37</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

* Approximate levels of Mn-SOD mRNA from Fig 1.
† Concentration that gave half-maximal binding of TNF-α. Results previously reported.22
‡ TNF concentration required to inhibit growth of half the colonies. Results previously reported.16

native HL-60 cells (Fig 2B). Time-course studies in native HL-60 cells showed that Cu/Zn-SOD RNA started to decrease 6 hours after addition of TPA (data not shown). These studies showed that either slowly or nondividing, myeloid cells that had undergone differentiation, had little change in their levels of Mn-SOD RNA.

Effect of TNF on RNA levels of Mn-SOD and other antioxidant enzymes in various hematopoietic cells. We evaluated the effect of TNF on a variety of normal and leukemic hematopoietic cells as well as human embryonic lung fibroblasts (Figs 3A through D and 4A and B). Human embryonic lung fibroblasts (Fig 3A), peripheral blood lymphocytes (PBL; Fig 3C) and monocytes (Fig 3D) expressed a low level of Mn-SOD RNA, whereas these cells exposed to TNF had a 5.3-, 5.3-, and 2.7-fold induction of Mn-SOD RNA, respectively. TNF induced a 3.8-fold increased level of Mn-SOD RNA in U937 (TNF-sensitive, Fig 3B). The HL-60R and K562 (both resistant to cytotoxic effects of TNF) constitutively expressed Mn-SOD RNA; and TNF did not increase levels of these transcripts (Fig 3A and B). In contrast, expression of GSX-PX transcripts in response to TNF decreased in PBL and were unchanged in U937 and K562 cells (Fig 3B and C).

Both dose- and time-response studies were performed to
analyze in greater detail the effects of TNF on the RNA and protein expression of antioxidant enzymes, using TNF-sensitive native (HL-60) and TNF-resistant (K562) cell lines. In native HL-60 cells, TNF induced both Mn-SOD RNA and Mn-SOD activity in a dose-dependent fashion (Figs 4A and 5). In these same cells, levels of Cu/Zn-SOD RNA slightly decreased and those of GSX-PX RNA also slightly decreased at 10^3 and 10^4 U/mL of TNF (Fig 4A). Time-dependent analysis showed that native HL-60 cells expressed greater than twofold higher levels of both Mn-SOD RNA and Mn-SOD activity within 30 minutes of exposure to TNF (10^3 U/mL) compared with control native HL-60 cells, and levels remained stable for at least 8 hours (Figs 4B and 5). The levels of both Mn-SOD RNA and Mn-SOD activity were initially high in TNF-resistant K562 cell, and did not change with exposure to TNF in dose- (Figs 4C and 5) and time-response (Figs 4D and 5) studies.

Antisense oligonucleotides to Mn-SOD enhanced TNF-mediated clonal inhibition of both K562 and native HL-60 cells. Incubation with antisense oligonucleotides to Mn-SOD significantly enhanced the clonal inhibition mediated by TNF for both the TNF-resistant (K562) and TNF-sensitive (native HL-60) cell lines as compared with clonal growth of these cells cultured with random oligonucleotides and TNF (P < .005) (Fig 6A and B). For example, K562 formed about 450 colonies. TNF alone (5,000 U/mL) or TNF plus random oligonucleotides had no effect on colony formation. However, TNF (5,000 U/mL) plus Mn-SOD antisense oligonucleotides decreased K562 colony formation about 67%. Also, TNF (25 U/mL) plus Mn-SOD antisense oligonucleotides decreased HL-60 colony formation by about 50% compared with plates having TNF plus random oligonucleotides, and about 60% compared with plates with TNF alone.

Fig 3. Effect of TNF on level of expression of RNAs for Mn-SOD and GSX-PX. Various normal and leukemic cells were treated with TNF (10^5 U/mL) for 8 hours. (A) WI38 (normal human embryonic lung fibroblasts) and HL-60R (TNF-resistant HL-60 cells). (B) U937 (monoblasts, TNF-sensitive) and K562 (myeloid/erythroid blast cells, TNF-resistant). (C) PBL. (D) Human monocytes. RNA analysis was performed as described in Materials and Methods. Each blot was hybridized with ^32P-labeled Mn-SOD, GSX-PX, and β-actin DNAs.
Fig 4. Northern analysis of RNA from native HL-60 (TNF-sensitive) and K562 (TNF-resistant) cells exposed in a dose- and time-response fashion to TNF. (A) Native HL-60 and (C) K562: Cells were exposed to increasing concentrations of TNF (0-10^4 U/ml) for 8 hours and evaluated by Northern analysis using ^32P-labeled Mn-SOD, Cu/Zn-SOD, GSX-PX, and ^beta-actin cDNAs. (B) Native HL-60 and (D) K562: Cells were cultured with TNF (10^3 U/ml) for 0 to 8 hours. Total RNA was isolated and Northern analysis was performed as described in Materials and Methods.

The Mn-SOD antisense oligonucleotides entered the cells, the Mn-SOD oligonucleotides were radiolabeled and incubated with native HL-60 cells (Fig 7A). Denaturing gel electrophoresis of the labeled oligonucleotides from the extracellular medium and in the cytoplasm of the cultured cells demonstrated that the oligonucleotides were stable in the culture medium, and entered and were stable for ≥36 hours in the cells.

We used the technique of RT-PCR analysis to demonstrate the effects of antisense oligonucleotides on the expression of Mn-SOD RNA in the cells. As shown in Fig 7B, Mn-SOD antisense oligonucleotides substantially reduced Mn-SOD RNA levels (lane 2). ^beta-actin was amplified simultaneously and showed comparable amounts of RNA were present in cells not containing (lane 1) and containing (lane 2) the Mn-SOD antisense oligonucleotides.

DISCUSSION

In this study we investigated both expression of Mn-SOD as well as Cu/Zn-SOD and GSX-PX in normal and leukemic cells, and the relationship between Mn-SOD expression and TNF cytotoxicity in myeloid leukemic cells. We found by Northern blot analysis that a wide variety of human hematopoietic cell lines blocked at different stages of differentiation (myeloblasts, promyelocytes, myelomonocytes, monoblasts, and erythroblasts) as well as normal human
lymphocytes and macrophages expressed the 1.0 kb and the alternatively spliced 4.0 kb Mn-SOD RNA. Also, Cu/Zn-
SOD and GSS-PX RNAs were expressed in these cells. Lev-
els of Mn-SOD activity correlated with levels of expression
of Mn-SOD RNA. Slowly or nondividing, terminally differ-
entiated HL-60 granulocytes and macrophages expressed
RNA coding for Mn-SOD at a level nearly equivalent to
native HL-60 cells.

Some cell types are sensitive and others are resistant to
the cytotoxic/cytostatic effects of TNF; how the antiproli-
The effects of Mn-SOD antisense oligonucleotides on the expression of Mn-SOD RNA. (A) Cellular uptake and stability of end-labeled–antisense Mn-SOD oligonucleotides. The 21 base nucleotide was incubated in PBS (lane 1), in culture medium with heat-inactivated serum (lane 2), and in the presence of native HL-60 cells for 36 hours at 37°C. The extracellular medium was removed and the cell lysed. The cell lysates (cytoplasmic fraction) and extracellular fractions were ethanol precipitated, and the oligonucleotides loaded onto lanes 3 and 4, respectively. (B) Specific reduction of Mn-SOD RNA in Mn-SOD antisense oligonucleotides-treated native HL-60 cells. Native HL-60 cells were incubated either with or without Mn-SOD antisense oligonucleotides and then total RNA was extracted. One-microgram of RNA was reverse transcribed to cDNA and amplified by PCR with primer pairs specific for Mn-SOD and β-actin as an internal control; the amplification products were subjected to PAGE and stained with ethidium bromide, which resulted in 350 bp and 853 bp (Mn-SOD) and β-actin) bands. Lane 1: untreated native HL-60 cells. Lane 2: Mn-SOD antisense oligonucleotides cultured native HL-60 cells. Bottom lanes show the results of Southern blot hybridization of the PCR products, with probes specific for Mn-SOD (upper bands) and β-actin genes (lower bands).

The susceptibility of cells to effects of TNF...
probably can be explained only by intracellular levels of Mn-SOD. Several studies have shown the importance of GSX-PX^2 and intracellular levels of glutathione in determining resistance to superoxide and/or TNF. Our laboratory found that clonal hematopoietic progenitor cells of transgenic mice having increased expression of Cu/Zn-SOD were resistant to TNF. Recently, we also showed that myeloid colony-forming cells that were incapable of making superoxide were resistant to TNF. TNF probably increases levels of superoxide in hematopoietic cells, which can rapidly injure these cells unless they are protected by one of several antioxidant enzyme pathways.

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Regulation of manganese superoxide dismutase and other antioxidant genes in normal and leukemic hematopoietic cells and their relationship to cytotoxicity by tumor necrosis factor

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