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Regulation of manganese superoxide dismutase in glomerular epithelial cells: Mechanisms for interleukin 1 induction

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Regulation of manganese superoxide dismutase in glomerular epithelial cells: Mechanisms for interleukin 1 induction. Reactive oxygen species have been implicated as mediators of tissue injury in glomerular inflammation. The expression of the antioxidant enzyme, manganese superoxide dismutase (MnSOD), was examined in primary cultures of rat glomerular epithelial cells (GEC) in response to inflammatory mediators. The results demonstrate that GEC respond to interleukin-1 (IL-1) and bacterial lipopolysaccharide (LPS) with an increase in MnSOD steady-state mRNA levels. The IL-1α-mediated induction of MnSOD mRNA levels was both time- and dose-dependent. Maximal levels, approximately 40-fold above controls, were observed at 12 hours with 2 ng/ml of IL-1α. MnSOD protein levels were also markedly elevated by IL-1α. The induction of MnSOD mRNA by IL-1α required *de novo* transcription as well as some degree of protein synthesis. To elucidate the potential intracellular signal that mediates IL-1α-dependent MnSOD expression, three classical signaling pathways were examined. We found no evidence that MnSOD induction by IL-1α is mediated by either the cyclooxygenase or lipoxygenase pathway or via activation of protein kinase C. Based on the presence of IL-1α in several forms of glomerular inflammation, the observed increase in MnSOD expression by this immunoregulatory cytokine must have an important role in the antioxidant defense of glomerular epithelial cells.

The renal glomerular epithelial cell (GEC) is an integral component of the three-layered ultrafiltration barrier of the glomerular capillary. This cell is affected in several forms of human and experimental glomerulonephritis. Morphological changes of the GEC and impairment of filtration are observed in glomerular diseases such as minimal change disease and most types of glomerulonephritis, and in a variety of experimental models of renal injury, such as puromycin and adriamycin nephrosis, and Heymann nephritis [1].

Reactive oxygen species (ROS) have been recognized increasingly as potential mediators of inflammatory cell injury during glomerulonephritis [2]. The inherent cellular protection against ROS consists of a distinct antioxidant system. The superoxide dismutases are the enzymes which detoxify the primary product of ROS generation, the superoxide anion radical, leading to the production of hydrogen peroxide. This reactive oxygen species is detoxified through the action of catalase or glutathione peroxidase [3].

It has also been observed that with glomerular inflammation there is increased expression of the inflammatory cytokines, interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) [4–9]. Recently, we and others have found a correlation between the action of these cytokines and the expression of the mitochondrial antioxidant enzyme, manganese superoxide dismutase (MnSOD). Those data demonstrated that steady-state MnSOD mRNA levels are induced following treatment with IL-1 and TNFα, as well as with bacterial lipopolysaccharide (LPS) in various cell lines, whereas mRNA levels for the cytosolic copper/zinc superoxide dismutase (Cu/ZnSOD), catalase, and glutathione peroxidase were unaltered in response to these same stimuli [10–12]. However, the induction of MnSOD by these inflammatory mediators is not observed in all cell types. In hepatocytes, for example, only interleukin 6 induced an increase in MnSOD mRNA levels [13]. In addition, Fujii and Taniguchi [14] have demonstrated that MnSOD levels are not elevated by TNF, IL-1, or LPS in TNF-sensitive human tumor cell lines.

To better understand the antioxidant system of the glomerulus, the current study examined the effects of several proinflammatory mediators on MnSOD and Cu/ZnSOD mRNA levels in primary cultures of the rat GEC. Increased steady-state MnSOD mRNA levels were observed following treatment with IL-1α and β, and LPS, but not in response to TNFα and IL-6, thus further demonstrating cell-specific MnSOD regulation. Since the signal transduction mechanism by which IL-1 induces MnSOD gene expression is not yet understood, we also evaluated the possible role of membrane phospholipid metabolites and protein kinase C (PKC) in the intracellular signaling of IL-1.

Methods

Glomerular epithelial cell culture

Primary cultures of the rat GEC were initiated according to established protocols [15]. Briefly, kidneys were removed from 150 to 200 g male Sprague Dawley rats, and cortices were dissected and diced with a razor blade. The minced cortices were passed sequentially through 250 μ, 106 μ, and 75 μ stainless steel sieves to isolate glomeruli. After 30 minutes of treatment with 180 U/ml collagenase (Worthington Biochemical Corp., Freehold, NJ, USA), glomeruli were suspended in K1–3T3 (50:50; vol/vol) medium [15] and plated onto 100 mm Petri dishes (Becton Dickinson, Lincoln Park, NJ, USA) coated with a 3 mm thick type

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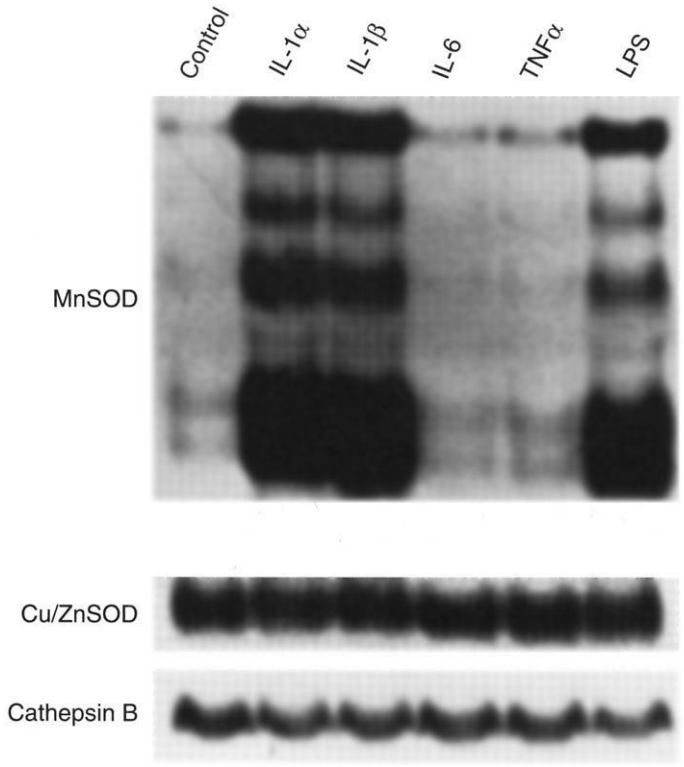


Fig. 1. *Effect of different inflammatory mediators on MnSOD mRNA and Cu/ZnSOD mRNA levels.* Glomerular epithelial cells were treated for 12 hours with 2 ng/ml IL-1α or β, 100 U/ml IL-6, 10 ng/ml TNFα, or 0.5 μg/ml LPS. The Northern blot was probed with a MnSOD cDNA, Cu/ZnSOD cDNA, and with a cathepsin B cDNA. mRNA levels were quantified densitometrically relative to the intensity of the cathepsin B signal.

I collagen matrix layer. The primary outgrowth observed after six days was excised with a razor blade, and transferred to 24-well culture dishes also coated with collagen. Further subcultures were performed in culture vessels of gradually increased size. For all subcultures, cells were rinsed with Ca^{++}, Mg^{++} free HBSS (Sigma, St. Louis, MO, USA), collagenase-treated 180 U/ml for 30 minutes to dissolve the collagen matrix, trypsinized with 0.05% trypsin (Sigma) in 0.5 mM EDTA Ca^{++}, Mg^{++} free HBSS for five minutes, resuspended in K1–3T3 culture medium and plated on the collagen matrix. For experiments, cells were plated on 100 mm Petri dishes and were studied after they formed a confluent layer. Passages 8 to 15 were used for all experiments.

Growth characteristics of the GEC were determined and the seeding efficiency was found to be 35%, with a population doubling time of 24 hours. Cells grew poorly in media lacking conditioned Swiss 3T3 fibroblast medium and permanent culture of the GEC was not possible without the use of a collagenous substrate.

The cells were characterized according to established criteria [15, 16]. Cells exhibited a polygonal shape and a cobblestone-like appearance at confluency. Immunohistochemical staining was positive with an antibody against cytokeratin (Amersham Corp., Arlington Heights, IL, USA), and was diffusely positive with an antibody against myosin (Biogeninex, Dublin, CA). The cells were negative for markers of other glomerular cell types such as factor VIII endothelial antigen (von Willebrand's factor; Immunon, Detroit, MI), common leukocyte antigens (LCA, Dako-LC, Dako Corp., Carpintesia, CA; and T29/33, Hybritech, San Diego, CA, USA), and actin (Enzo Diagnostics, Inc., New York, NY, USA). It is currently not possible to determine specifically whether GEC in culture originate from the visceral or parietal epithelium [1]. However, since the majority of glomeruli isolated from the tissue are decapsulated after sieving [15], we believe the cells are most likely of visceral origin.

Experimental stimuli and inhibitors

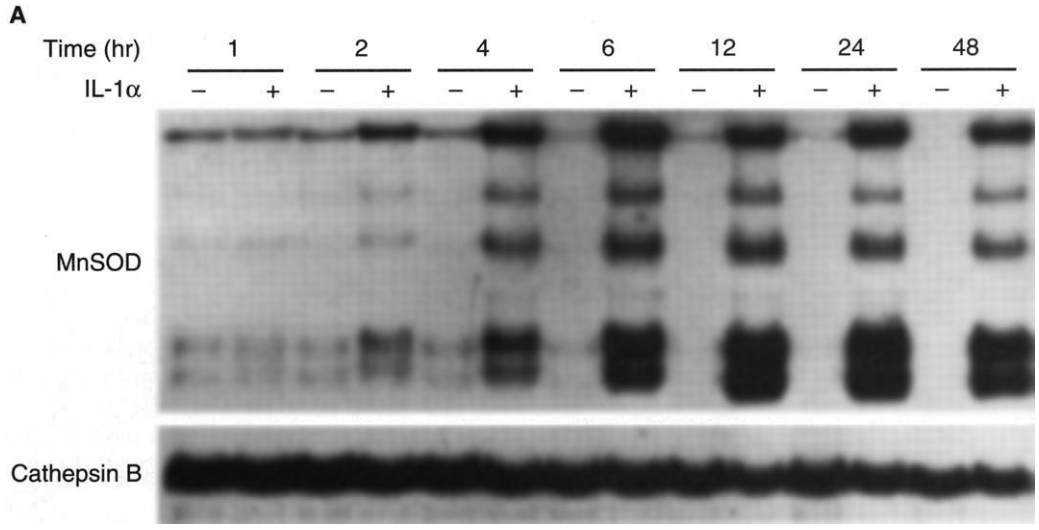
The cytokines utilized were all human recombinant products. IL-1 α and β were obtained from an NCI-sponsored program. TNFα was a gift from Genentech (Ca, USA); IL-6 was a gift from Genetics Institute (Cambridge, MA, USA). Bacterial lipopolysaccharide (*E. coli* endotoxin L2637, Sigma) as well as the cytokines were dissolved in sterile water and added to the cell culture plates at the dosages indicated in the **Results** section. The phorbol ester, 12-O-tetradecanoylphorbol 13-acetate (TPA), was dissolved in dimethyl sulfoxide (DMSO) (both from Sigma) at varying concentrations in that a constant volume of TPA solution, 10 μl/10 ml medium, was required to give the desired final TPA concentration in the culture medium as indicated in the **Results** section. Actinomycin D at a concentration of 4 μM and cycloheximide at a concentration of 20 μM (Sigma) were employed to inhibit RNA synthesis and protein synthesis, respectively.

Indomethacin, nordihydroguaiaretic acid (NDGA) and 1-phenyl-3-pyrazolidone (Sigma) were dissolved in absolute ethanol. 5-,12-,15-(S)-Hydroxyeicosatetraenoic acid and the leukotrienes, LTB4, LTC4, LTD4, were obtained from Sigma, and the 5-,12-, 15-(S)-Hydroperoxyeicosatetraenoic acid from Cayman Chemical (Ann Arbor, MI, USA).

Evaluation of steady-state mRNA levels

RNA isolation and Northern blotting. Total cellular RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction method [17]. Samples of 15 μg of total cellular RNA were dissolved in 25 μl of buffer containing 50% (vol/vol) formamide, 6.4% formaldehyde, 20 mM/liter morpholinepropanesulfonic acid (MOPS), 6 mM/liter sodium acetate, and 0.5 mM/liter EDTA (pH 7.0). RNA samples were denatured at 65° for 15 minutes and 5 μl of a 1.2 mM/liter ethidium bromide solution was added. The RNA was fractionated by size on a 1% agarose, 6% formaldehyde gel. The RNA was electrotransferred to a noncharged nylon membrane (Genescreen, DuPont-New England Nuclear, Boston, MA, USA) and covalently cross-linked to the membrane with UV light [10].

Hybridization with cDNA probes. The membrane was prehybridized in 0.45 M sodium phosphate, 7% sodium dodecyl sulfate, 1 mM EDTA, and 1% bovine serum albumin for 20 minutes. The membrane was hybridized for 16 hours at 60°C in the above hybridization solution with a ^{32}P-labeled rat MnSOD cDNA, a rat Cu/ZnSOD cDNA, rat preprocathepsin B cDNA (a gift from S.J. Chan, University of Chicago), or a rat c-*fos* cDNA (a gift from T. Curran, Roche Institute, Nutley, NJ, USA). The two rat cDNA probes for MnSOD and Cu/ZnSOD were 936 and 650 base pairs, respectively. They have been isolated in our laboratory and will be described in more detail elsewhere. Northern analysis using primarily coding sequence from a MnSOD cDNA demonstrated the presence of five transcripts (Fig. 1). Based on studies in our



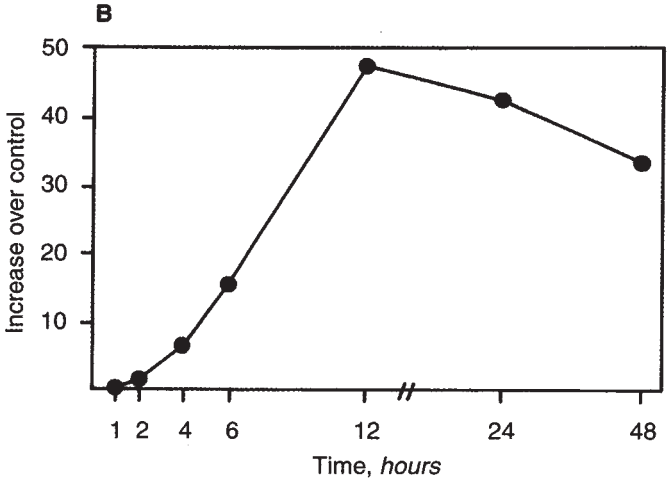


Fig. 2. A. *Time-dependent increase of MnSOD mRNA levels in the GEC following treatment with 2 ng/ml IL-1α.* **B.** Quantitative summary of these experiments. The MnSOD mRNA levels of treated cells are expressed as *N*-fold increases above the mRNA levels of the untreated controls. $N = 3$.

laboratory, we have shown that these transcripts represent alternatively polyadenylated products [18].

Densitometry. Cu/ZnSOD, MnSOD, and preprocathepsin B mRNA levels were quantified using the Bio Image Visage 60 video densitometry system (Millipore/Bio Image, Ann Arbor, MI, USA). Densitometry for quantitation of the autoradiographs was performed only on the two most abundant transcripts. For all experiments, quantitative data were presented only on signals in the linear range of the film and the densitometer. Individual lanes were compared with respect to the intensity of the preprocathepsin B signal, that is, the data are expressed as a ratio of MnSOD/preprocathepsin B. Numbers represent the mean ± SD, where given, from at least three independent experiments, unless otherwise stated.

Detection of MnSOD protein

Cells were harvested at specific time intervals following IL-1α treatment together with their appropriate controls. Cell culture medium was not exchanged between IL-1α application and cell harvesting. To harvest cells, plates were collagenase-treated as described in GEC culture, washed four times with ice-cold 20 ml

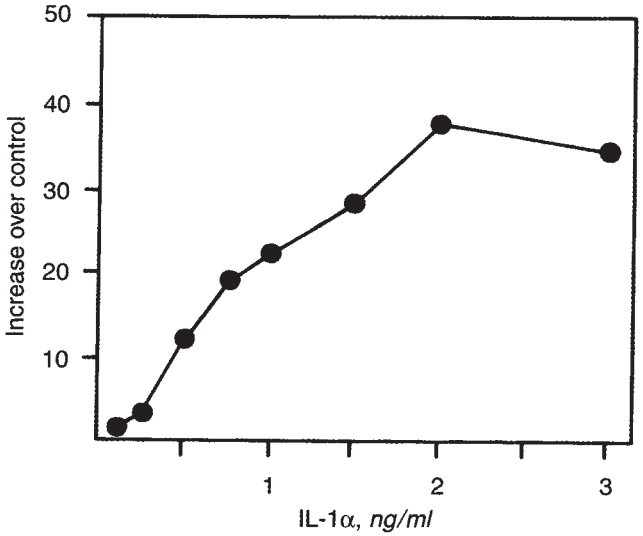


Fig. 3. *Densitometric data on the dose-dependent induction of MnSOD mRNA levels in glomerular epithelial cells treated with IL-1α for 12 hours relative to the untreated controls.* $N = 2$.

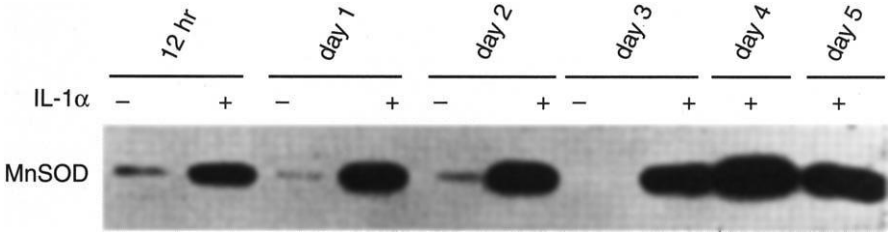


Fig. 4. *Time-dependent accumulation of MnSOD protein in glomerular epithelial cells treated with 2 ng/ml IL-1α.*

HBSS and resuspended in 0.5 ml of a pH 7.5, 0.25 M sucrose, 10 mM Tris, 1 mM EDTA solution. Then, 20 μM phenylmethyl sulfoxide and 10 μg/ml protamine were added to the samples. Samples were incubated with 2% Triton X-100 for five minutes, homogenized, and centrifuged at 1000 rpm for five minutes at 4°C. The total protein content was determined in the supernatant [19] and samples of 10 μg protein were applied to a SDS/15% acrylamide gel for separation of proteins under reducing conditions [20]. The samples were blotted to nitrocellulose using the buffer system described by Towbin, Staehelin and Gordon [21]. The membrane was probed with a 1:1000 dilution of rabbit polyclonal antibody against rat MnSOD [22]. The bound primary antibody was detected with the enhanced chemiluminescence kit from Amersham Corp.

Results

The response of the GEC to specific cytokines and LPS is depicted in Figure 1. Untreated cells exhibited low steady-state MnSOD mRNA levels, whereas IL-1α and β caused a 49-fold and 35-fold increase, respectively, in MnSOD mRNA when compared to the untreated controls. LPS increased MnSOD mRNA levels by 18-fold. Unlike glomerular mesangial cells, the GEC did not respond to TNFα (Fig. 1), even when used at doses as high as 100 ng/ml (Nick and Gwinner, unpublished observations). In addition, IL-6 did not change MnSOD mRNA levels.

To determine the effect of these stimuli on Cu/ZnSOD mRNA levels in the GEC, the membrane was reprobed with the corresponding cDNA. As shown in Figure 1, none of the stimuli altered Cu/ZnSOD mRNA levels. We have found that preprocathepsin B (cathepsin B) mRNA levels in the GEC are constant under most experimental conditions. Therefore, loading variations and densitometric quantitations were determined relative to cathepsin B mRNA levels.

Because of the importance of IL-1 in glomerular inflammation, we concentrated our efforts on the effects of IL-1α on MnSOD mRNA induction. Figure 2 A and B demonstrate that the induction of MnSOD mRNA levels by IL-1α is time-dependent with a maximal response at 12 hours, although some variability was observed among the five transcripts. The induction of MnSOD mRNA was also dose-dependent as depicted in Figure 3. In these 12 hour experiments, maximal induction was seen at a concentration of 2 ng/ml IL-1α, but a dose as low as 0.25 ng/ml, corresponding to 15 pM, was still effective. Based on these results with IL-1α, a dose of 2 ng/ml was utilized for the subsequent studies.

Figure 4 illustrates the effect of IL-1α on MnSOD protein levels at specific time points after treatment, as determined by Western analysis. At 12 hours the protein levels were maximal and remained high throughout the entire observation period of five days.

To determine whether the induction of MnSOD mRNA by IL-1α required new transcription, cells were co-treated with IL-1α and the transcriptional inhibitor, actinomycin D, at a dose of 4 μM (Fig. 5A). Actinomycin D abolished the IL-1α-mediated increase in MnSOD mRNA, indicating that this induction is dependent on *de novo* mRNA synthesis.

Cells were also co-treated with IL-1α and the protein synthesis inhibitor, cycloheximide. As depicted in Figure 5B, cycloheximide did not prevent the IL-1α-dependent increase. However, the MnSOD mRNA levels were lower when compared to treatment with IL-1α alone. These results indicate that new protein synthesis is not a strict prerequisite for the IL-1α-dependent induction of MnSOD mRNA. The reduced level of MnSOD mRNA observed after the co-treatment with IL-1α and cycloheximide could indicate a requirement for protein synthesis for maximal MnSOD mRNA induction. Furthermore, we interpret the higher cathepsin B mRNA level observed in cycloheximide-treated cells as an actual increase in cathepsin B mRNA levels presumably due to mRNA stabilization by cycloheximide, especially since the ethidium bromide staining indicated comparable loading in every experiment.

There are several potential intracellular signaling pathways for IL-1 action including: phospholipase A_2 activation resulting in the release of arachidonic acid metabolites as second messengers [23, 24]; activation of phospholipase C [25]; and/or activation of protein kinase C (PKC) [26]. In an attempt to elucidate the intracellular signaling mechanism by which IL-1α induces increased MnSOD mRNA levels, the involvement of cyclooxygenase or lipoxygenase-derived arachidonic acid metabolites in the IL-1α mediated MnSOD induction was evaluated. Cells were treated with either 50 μM indomethacin to inhibit the cyclooxygenase, or 20 to 30 μM nordihydroguaiaretic acid (NDGA) to inhibit lipoxygenases, followed by the addition of IL-1α for 6 or 12 hours. Compared to IL-1α treatment alone, treatment with indomethacin did not inhibit the IL-1α-mediated induction of MnSOD mRNA levels (data not shown). Treatment with NDGA at both 20 μM and 30 μM failed to decrease the IL-1α-induction of MnSOD mRNA which is demonstrated for the 12-hour incubation period in Figure 6. In addition, another lipoxygenase inhibitor, 1-phenyl-3-pyrazolidone (50 μM), also showed no inhibition on the IL-1α-mediated MnSOD induction (data not shown).

In another study, Haliday, Ramesha and Ringold [27] demonstrated that TNFα, which shares many similar biological effects with IL-1, induced the protooncogene, c-*fos*, in adipocytes via metabolites of arachidonic acid. The TNFα-mediated c-*fos* induction could be blocked by lipoxygenase inhibitors, whereas the lipoxygenase products, 5-,12-, or 15-hydroperoxyeicosatetraenoic acid (HPETES), could mimic the increase in c-*fos* mRNA. Therefore, we examined a number of lipoxygenase metabolites for

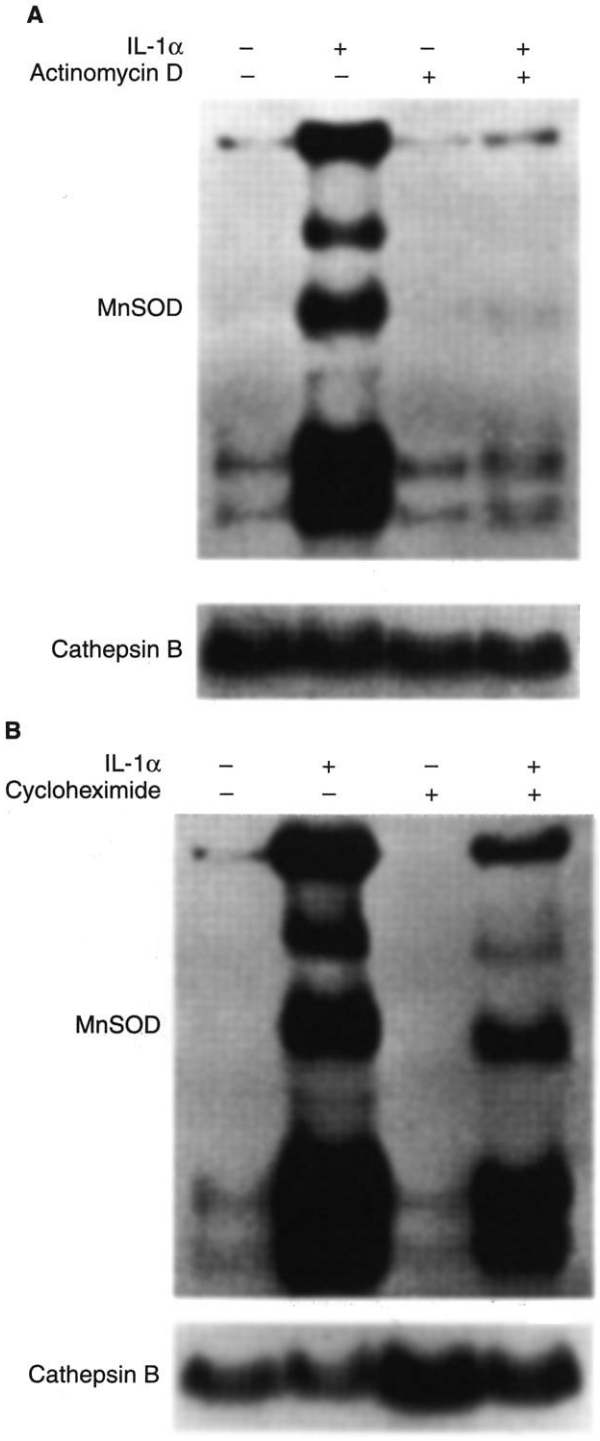


Fig. 5. *Effect of the transcriptional inhibitor, actinomycin D (A), and the protein synthesis inhibitor, cycloheximide (B) on the IL-1α-mediated MnSOD mRNA induction.* Glomerular epithelial cells were treated for 12 hours with 2 ng/ml IL-1α alone or co-treated with IL-1α and 4 μM actinomycin D or 20 μM cycloheximide, respectively.

their ability to mimic IL-1-dependent MnSOD expression including 5-,12-, and 15-HPETES as well as the more stable metabolites, 5-,12-, and 15 hydroeicosatetraenoic acid (HETE); and the leukotrienes B4, C4, and D4. Because of the potential instability of these lipids, incubation periods of two and six hours were selected. In the case of 5-,12-, and 15-HPETES which are radicals, one and four hour time points were also examined. Using concentrations of 0.1 μM and 1.0 μM of 5-,12-, and 15-HETE, 5-,12-, and 15-HPETE, and of the different leukotrienes, no changes in MnSOD mRNA levels were observed at any time point (data not shown).

To examine the potential role of protein kinase C (PKC) activation in the IL-1α-mediated induction of MnSOD mRNA levels, PKC activity was down-regulated in the GEC by the phorbol ester TPA. It is well established that short-term treatment with TPA induces PKC activity and that high doses over a prolonged period deplete most types of PKC activity [28]. Therefore, we first examined whether an 18-hour treatment with 2.4 μM TPA was sufficient to down-regulate PKC. In these experiments, c-*fos* mRNA levels were used as an indicator of PKC activity, because it has been shown that c-*fos* mRNA can be induced by PKC activation [28]. Figure 7 demonstrates that stimulation of PKC by 0.4 μM TPA causes a rapid and transient induction of c-*fos* mRNA at 0.5 hours with a maximum at one hour (left panel). However, following an 18 hour pre-treatment with 2.4 μM TPA, c-*fos* mRNA could not be induced by 0.4 μM TPA (right panel), indicating a significant down-regulation of PKC activity. Figure 7 also depicts the same blot reprobed with MnSOD cDNA. As seen in the left panel, treatment with 0.4 μM TPA caused a sixfold induction of MnSOD mRNA levels within six hours, similar to results recently shown in pulmonary cells [29]. Lower doses of TPA between 10 to 150 nM were also capable of inducing MnSOD mRNA levels (data not shown), but 0.4 μM exhibited the greatest and most consistent response. In comparison to the rapid increase and destabilization of c-*fos* mRNA following TPA treatment, the MnSOD induction was delayed and elevated levels were sustained for six hours. Furthermore, elevated MnSOD levels were even observed after 18 hours of pretreatment with 2.4 μM TPA, as shown in the control lane in the right panel of Figure 7. Following re-exposure of TPA pretreated cells to 0.4 μM TPA, no further increase in MnSOD mRNA levels was observed, suggesting a possible role for PKC activation in MnSOD gene activation by TPA.

We next examined the involvement of PKC activation in the IL-1α-mediated induction of MnSOD mRNA. The left and middle panel of Figure 8A shows either control cells or cells pretreated with the vehicle, respectively, in combination with the various stimuli. As is evident from the right panel of Figure 8A and the quantitative summary in Figure 8B, PKC down-regulation by TPA did not change the ability of IL-1α to induce MnSOD mRNA levels in these cells. Similar results were obtained for LPS, indicating that neither IL-1α nor LPS-mediated MnSOD induction is dependent on PKC activation.

Discussion

ROS have been implicated increasingly as mediators in tissue injury in several forms of inflammatory glomerular disease [2]. The likely mechanism of this injury is based on the damage of cellular components such as DNA, cellular proteins and lipids by ROS, ultimately resulting in cell dysfunction and death [30].

The present study focuses on the regulation of the superoxide dismutases, the primary cellular defense against ROS, in cultured GEC. We found marked stimulation of MnSOD mRNA steady-state levels in the GEC after treatment with either IL-1α, β, or

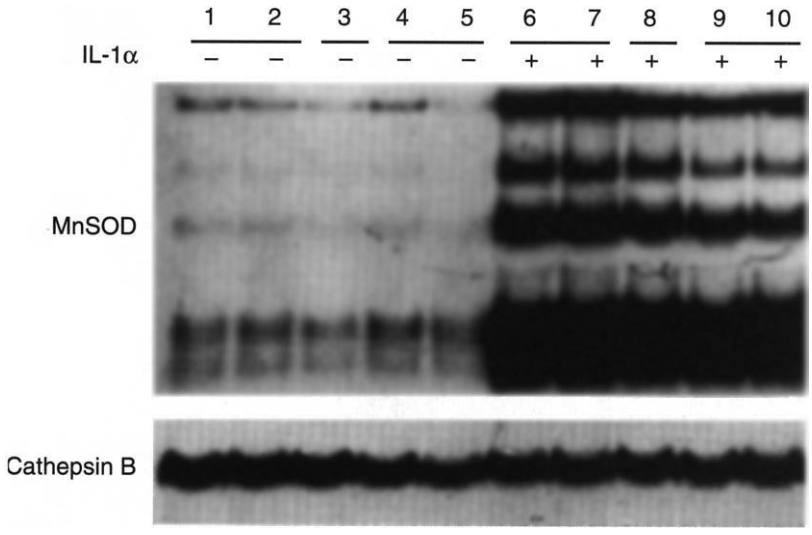


Fig. 6. *Effect of lipoxygenase inhibition on IL-1α-mediated MnSOD mRNA induction.* Glomerular epithelial cells were treated for 30 minutes with the vehicle (lanes 1 and 2), ethanol (lanes 3 and 8), 20 μM NDGA (lanes 4 and 9), and 30 μM NDGA (lanes 5 and 10). Subsequently, the cells were incubated for 12 hours with 2 ng/ml IL-1α as indicated by (+). Lanes 6 and 7 were treated only with IL-1α to demonstrate the usual level of MnSOD induction.

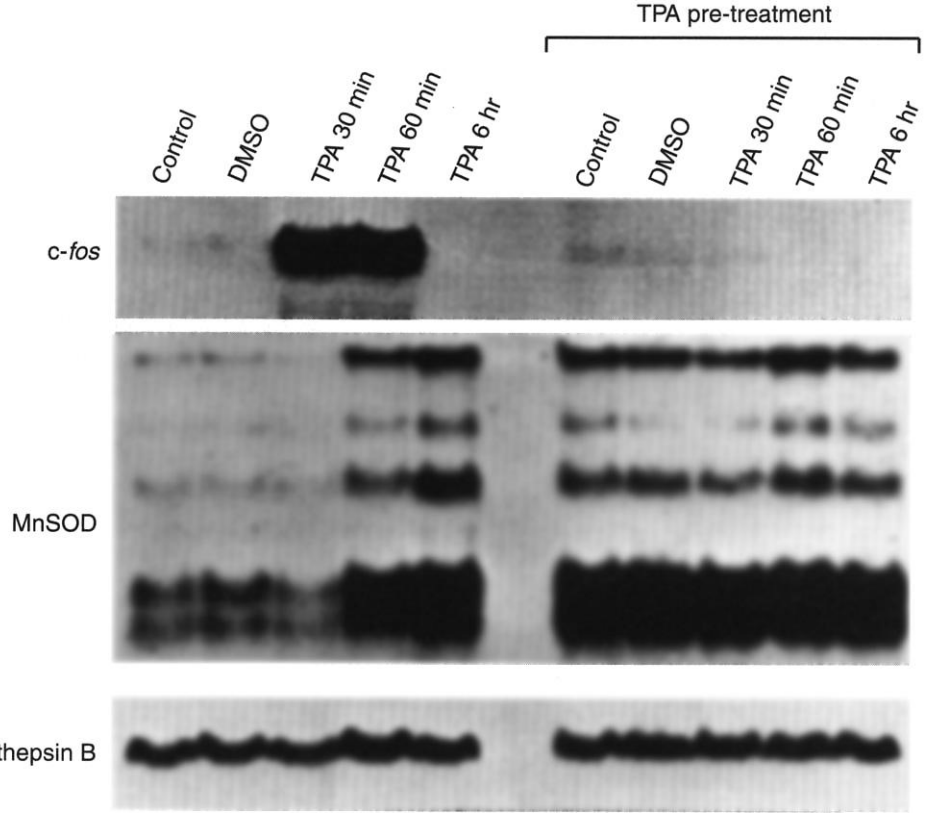


Fig. 7. *Illustration of PKC down-regulation in the GEC as demonstrated by the inability to induce c-fos mRNA with 0.4 μM TPA (right panel) as opposed to the c-fos mRNA induction by 30 or 60 minutes treatment with 0.4 μM TPA in cells without prior PKC down-regulation (left panel).* PKC down-regulation was achieved by 18 hours of pretreatment with 2.4 μM TPA. The same blot reprobed with the MnSOD cDNA shows a time-dependent increase in MnSOD mRNA levels after TPA treatment.

LPS. Unlike mesangial cells, GEC did not respond to the inflammatory mediator, TNFα. This observation is also in contrast to results in other epithelial cell lines originating from the lung [10] and small intestine [11], where TNFα caused large increases in MnSOD mRNA levels. Similar to the IL-6-specific induction of MnSOD in hepatocytes [13], our results in glomerular epithelial cells are therefore another example of the cell type-specific regulation of MnSOD. In agreement with previous observations, none of the stimuli increased Cu/ZnSOD mRNA levels [10–12], indicating that at least under these circumstances the constitutive

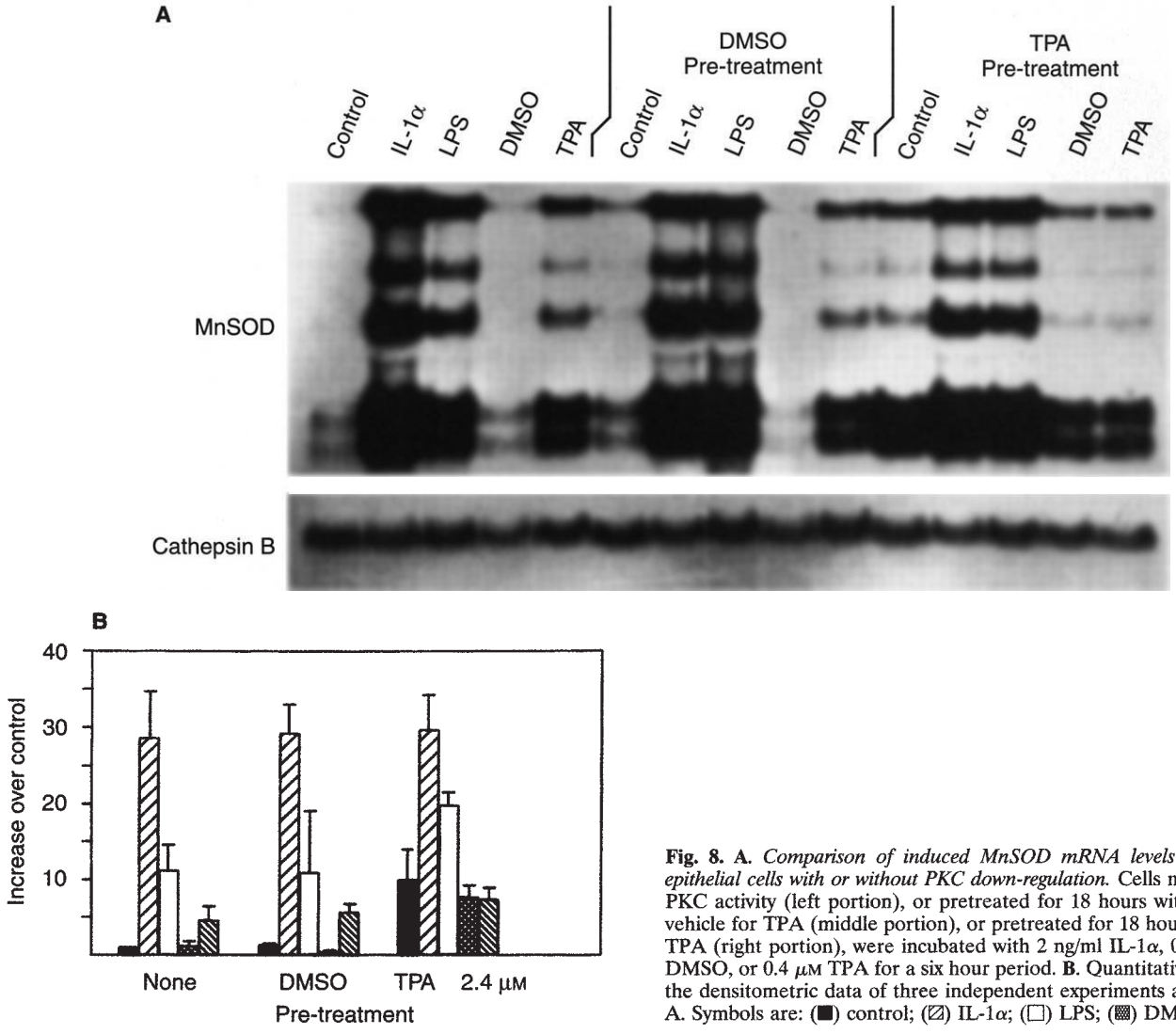


Fig. 8. A. *Comparison of induced MnSOD mRNA levels in glomerular epithelial cells with or without PKC down-regulation.* Cells not depleted of PKC activity (left portion), or pretreated for 18 hours with DMSO, the vehicle for TPA (middle portion), or pretreated for 18 hours with 2.4 μM TPA (right portion), were incubated with 2 ng/ml IL-1α, 0.5 μg/ml LPS, DMSO, or 0.4 μM TPA for a six hour period. **B.** Quantitative summary of the densitometric data of three independent experiments as described in A. Symbols are: (■) control; (▨) IL-1α; (□) LPS; (▩) DMSO; (▧) TPA.

expression of this antioxidant enzyme may be adequate for its protective role in the cytoplasm.

Several observations support an important role for IL-1 in inflammatory glomerular disease. IL-1 expression in kidney tissue is enhanced in experimental immune complex nephritis [4], puromycin nephrosis [5], anti-GBM nephritis [6], lupus nephritis [7], and in glomerular cell cultures from patients with rapidly progressive crescentic glomerulonephritis [8]. Therefore, further studies were performed using IL-1α. The IL-1α-mediated increase in MnSOD mRNA was both dose- and time-dependent. Furthermore, we have also demonstrated the time-dependent accumulation of MnSOD protein following IL-1α treatment in the GEC. With regard to the physiological significance of this response, *in vitro* observations provide evidence that elevated SOD protein levels confer resistance against ROS damage in mammalian cells and, conversely, lack of inducible SOD genes rendered the cells more sensitive to oxidative damage [31, 32]. In addition, *in vivo* experiments with transgenic mice, expressing enhanced MnSOD protein levels, demonstrated protection against otherwise lethal hyperoxia [33]. In the kidney, glomerular antioxidative enzymes including MnSOD are moderately induced by steroids and this induction conferred protection in puromycin aminonucleoside nephrosis [34].

The mechanism by which IL-1 induces an increase in MnSOD in mRNA is not yet understood. However, since IL-1 has been shown to interfere with mitochondrial respiration by inhibiting mitochondrial aconitase [35, 36], the potential importance of the regulation of MnSOD that is localized specifically to mitochondria is more easily understood. Our results in GEC co-treated with IL-1α and actinomycin or cycloheximide indicate that *de novo* mRNA transcription is required as well as some degree of new protein synthesis. This is further supported by nuclear run-on studies which demonstrate that MnSOD gene transcription is enhanced in response to IL-1α (Hsu and Nick, unpublished observations). These data suggest that the induction of MnSOD gene expression by IL-1α occurs via the generation of an intracellular signal that may alter a pre-existing transcription factor, as has been described for interferon α-induced effects [37]. The

transcription factor then presumably enhances transcription by binding to the MnSOD promotor or by releasing a repressor molecule from the promotor.

To further characterize the mechanism controlling IL-1- mediated induction of MnSOD, we examined potential intracellular signaling pathways. Of the known pathways associated with IL-1-induced effects in different cell types, no single unifying mechanism has been identified. IL-1 has been shown to induce the production of the lipoxygenase metabolites 5-, and 15-HETE in T cells. Lipoxygenase activity was also found to be necessary in IL-1-mediated IL-2 production in these cells, as demonstrated by studies with a lipoxygenase inhibitor [24]. In addition, Muñoz et al [26] have recently demonstrated that IL-1-dependent induction of IL-5 in T cells is mediated by protein kinase C.

Therefore, we evaluated the potential role of lipoxygenase as well as cyclooxygenase metabolites as an intracellular signal for MnSOD expression in the GEC. With regard to our studies, these metabolites are synthesized by GEC [38, 39]. Our experiments with the cyclooxygenase inhibitor, indomethacin, indicate that cyclooxygenase metabolites are not necessary for the IL-1α induction of MnSOD. Also, the lipoxygenase inhibitor, NDGA, had no suppressive effect on the IL-1α induction of MnSOD mRNA levels. In addition, the cells were treated with a number of metabolites from the lipoxygenase pathway to evaluate whether these substances could mimic IL-1α in inducing the MnSOD mRNA levels. We were unable to demonstrate an increase in MnSOD mRNA following incubation with either 5-,12-, or 15-HETE, 5-,12, or 15-HPETE, or the leukotrienes, LTB4, LTC4, LTD4. Moreover, we could not detect any increase in c-*fos* mRNA at one hour following treatment with 5-, 12-, or 15-HPETE (data not shown) as observed in adipocytes by Haliday et al [27], even though c-*fos* mRNA can be induced by TPA in GEC (Fig. 8). In conclusion, our experiments with lipoxygenase metabolites do not provide evidence that these substances are involved in MnSOD gene induction in GEC.

As indicated above, activation of PKC may also be involved in the intracellular signaling pathway of IL-1 [26]. In addition, IL-1 increases the generation of diacylglycerol, a co-factor of PKC, from membrane phospholipids in a number of cell types [25, 40]. Therefore, we examined whether down-regulation of PKC activity would abolish the IL-1α-dependent induction of MnSOD mRNA levels. Our results in PKC-depleted GEC (Fig. 8A and 8B) demonstrate that PKC activity is not required for the IL-1α-mediated MnSOD mRNA induction. The induction of MnSOD mRNA observed after TPA-treatment may be an alternative means of MnSOD gene induction, presumably mediated by PKC as suggested recently by another group [14].

In conclusion, we have shown that GEC respond to IL-1 and LPS with a dramatic increase in MnSOD expression. The intracellular signaling mechanism of IL-1-mediated MnSOD induction presumably does not involve metabolites of the cyclooxygenase and lipoxygenase pathway, or PKC activation. These results suggest that IL-1 must employ a novel signaling pathway for the induction of this important mitochondrial-localized antioxidant enzyme. Based on the central role of IL-1 in glomerular inflammation, the IL-1-induced MnSOD expression in GEC we have demonstrated *in vitro* might have important implications for the cellular defense against ROS toxicity.

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